

# **VULVAL VESTIBULITIS SYNDROME**

A Thesis submitted for the Degree of Doctor of Medicine

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# **ABSTRACT**

## **Objective**

This project investigates ways of assessing Vulval Vestibulitis Syndrome (VVS), possible aetiological factors, and response to a range of treatments.

## **Materials and Methods**

1. Data were collected and analysed to identify possible epidemiological characteristics and compared to existing evidence.
2. Technology (an algometer) was used to reliably assess patients, and quantify response to treatment.
3. Immunohistochemistry was used to determine whether VVS is an inflammatory condition.
4. Immunohistochemistry was also used to investigate the expression of oestrogen and progesterone receptors in the vulval tissue of women with VVS and biochemical techniques were used to investigate any relationship with serum oestradiol.

## **Results**

The cohort of women fulfilling the criteria for VVS were aged 22 to 53 (mean age 34.6) and for some there appeared to be an association with use of the combined oral contraceptive pill (cOCP).

The algometer allowed quantification of pain, and improvement with treatment.

There appears to be an overall decreased expression of inflammatory markers in women with VVS.

There appears to be no correlation between serum oestradiol and VVS, however vulval tissue from women with VVS appears to express less oestrogen receptors, although there is no difference in the expression of progesterone receptors.

## **Discussion**

VVS is a condition which appears to have well defined epidemiological characteristics, although subgroups may exist.

There is evidence that it is not an inflammatory condition, as previously thought, giving weight to the current argument that it is not an “itis” and the condition should be defined as localised provoked vulvodynia, as proposed by the International Society for the Study of Vulvovaginal Disease (ISSVD) at their congress in 2003.

The nomenclature and classification of vulval pain forever appears to be evolving. Since late 2003 the term Vulval Vestibulitis Syndrome is no longer recommended by the ISSVD, although it was the term in use at the time this work was performed. The patients were selected for the studies by fulfilling Friedrich's criteria for VVS, and therefore this term is used throughout this thesis



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## **PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS THESIS**

Eva LJ, Reid WMN, Morrison G and MacLean AB

The use of the vulval algometer to assess response to treatment in vulval vestibulitis syndrome

American Journal of Obstetrics and Gynecology 1999;181(Vol1):99-102

Eva LJ

A GP's guide to managing vulval pain

Practitioner March 2000 244(1608): 225 228 - 33

Eva LJ and MacLean AB

The diagnosis and management of vulvodynia

Gynaecology Abstracts 2002 Vol 5:2-5

Eva LJ

Vulval disorders

Chapter in: Women's Health 2003

Oxford University Press Ed:D Waller and A McPherson

Eva LJ, MacLean AB, Reid WMN, Rolfe KJ and Perrett CW

Estrogen receptor expression in vulvar vestibulitis syndrome

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Eva LJ, Rolfe KJ, Reid WMN, Perrett CW, Wong te Fong AC and MacLean AB

Provoked localized vulvodynia:Not an inflammatory condition

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### **Abstracts**

Eva LJ, MacLean AB and Reid WMN

Oestrogen levels and Vulval Vestibulitis Syndrome

British Journal of Obstetrics and Gynaecology 1998 Vol 105 suppl.17 122 – 3

Eva LJ, Reid WMN and MacLean AB

Referral Patterns and outcome data of a cohort of patients with VVS

Journal of Obstetrics and Gynaecology 2000 Vol 20 (5): 558

Eva LJ, Rolfe KJ, Reid WMN, Perrett CW, Wong te Fong AC and MacLean AB

Provoked Vulvar Dysesthesia localized to the vestibule:Not an inflammatory condition

Journal of Reproductive Medicine 2004 Vol 49 (11):922

## **Oral presentations**

Provoked vulvar dysesthesia localized to the vestibule: not an inflammatory condition  
International Society for the study of Vulvovaginal Disease, Salvador Brazil, 2003

Expression of TNF alpha in Vulval Vestibulitis Syndrome  
British Society for the Study of Vulval Disease, Salisbury UK, 2002

Referral patterns and treatment outcome of a cohort of patients with vulval vestibulitis syndrome  
British Society for the Study of Vulval Disease, Manchester, 2000

Understanding vulvodynia  
RCOG meeting on vulval pain, London, 2000

Immunohistochemical analysis of oestrogen receptor expression in vulval vestibulitis  
British Congress of Obstetrics and Gynaecology, Cape Town, South Africa, 1999

The use of the algometer in vulval vestibulitis syndrome  
The Mediterranean Conference for female lower genital tract diseases, Eilat, Israel 1998

Oestrogen levels and vulval vestibulitis syndrome  
The Mediterranean Conference for female lower genital tract diseases, Eilat, Israel 1998

## **Poster presentations**

Use of the vulval algometer to assess response to treatment in vulval vestibulitis syndrome  
British Congress of Obstetrics and Gynaecology, Harrogate UK, 1998

Epidemiological survey of a cohort of patients with vulval vestibulitis syndrome  
British Society for the Study of Vulval Disease, Oxford, 1998

Cytokine expression in Vulval Vestibulitis Syndrome  
British Congress of Obstetrics and Gynaecology, Sydney Australia, 2002



## **CHAPTER ONE - INTRODUCTION**

### **Embryology of the vulva**

The vulva begins to develop in the third week of gestational development when the three germ layers, the ectoderm, mesoderm and endoderm, form in the embryo.

The cloacal membrane forms between the ectoderm and distal endoderm.

The primitive cloaca divides into the anterior urogenital portion and the rectal portion, separated by the urorectal septum. In the sixth week, the cloacal membrane breaks down anteriorly to leave the urogenital sinus, which is divided into three portions, the caudal end being the genital tubercle, from the fusion of the cloacal folds. The phallic portion of the urogenital sinus remains open and between 14 and 20 weeks the vagina opens into the pelvic portion, converting it into the vestibule.

The lateral genital swellings become visible and these ultimately evolve into the labia majora. The medial urethral folds develop into the labia minora.

The genital tubercle enlarges slightly to form the clitoris. (Langman 1990)

Presence of the XX chromosome and lack of Mullerian inhibiting substance allows the ovary to develop and it is thought that oestrogens produced by the primitive ovary stimulate the development of the external genitalia although this is still unclear (George 1988)

Differentiation between the sexes occurs by the tenth week.

## **Anatomy of the vulva**

The vulva comprises of the mons pubis, labia majora, labia minora, clitoris, vestibule of the vagina, bulb of the vestibule and the greater vestibular glands.

The anterior border of the vulva is the mons pubis which is the hair bearing skin and subcutaneous fat overlying the symphysis pubis. The lateral margins are the lateral aspects of the labia majora and the vulva is bounded posteriorly by the perineal body.

The labia majora extend posteriorly from the mons pubis and are the outer hair bearing folds of the vulva. Medial to these are the hairless folds of the labia minora. These join together posteriorly at the fourchette. Anteriorly they enclose the clitoris and the prepuce, the skin covering of the clitoris.

The labia majora merge into the perineum posteriorly.

The vulval vestibule is the area at the entrance to the vagina bounded by Hart`s line, the junction between two different types of skin. It extends caudally from the hymenal rim and is bounded posteriorly by the fourchette. It extends anteriorly to border the urethral meatus. This is shown in Fig.1.1.

Fig 1.1 The vestibule



There are two major glands opening into the vestibule, but Robboy (1978) described multiple openings appearing as tiny pits in the vestibular surface which may not be identified without colposcopic magnification.

Hunt (1942) makes reference to "glandulae vestibuli minores", but the main glands of the vestibule are best recognised as the two paraurethral (Skene`s) glands anteriorly, and the two major vestibular or Bartholin`s glands posteriorly.

Each of these glands lead to a duct approximately two centimetres long.

When in the lithotomy position these gland openings are visible at 5 and 7 o'clock.

These glands are lobulated and contain multiple acini, lined with cuboidal epithelium and the ducts are lined with stratified transitional epithelium. The glandular secretion is clear, mucoid and alkaline and increases with sexual arousal.

The blood supply of the vulva includes the superficial external pudendal artery and deep external pudendal artery, both of which are branches of the femoral artery. These provide the main arterial supply for the labia majora.

The internal pudendal artery is a branch of the internal iliac artery and supplies the clitoris and labia as well as the muscles of the perineum.

It enters the perineum by passing through the lesser sciatic foramen and travels forwards with the pudendal nerve in Alcock's canal when it branches to supply the various aspects of the vulva.

Also contributing to blood supply is a branch of the inferior epigastric artery.

The venous drainage of the vulva is via veins accompanying the arteries described above.

The lymphatic drainage of the vulva is via lymph vessels which accompany the vessels. Drainage is to the superficial and deep inguinal groups of nodes.

The nerve supply of the vulva is derived from the roots of L1 and L2 via the genitofemoral nerve and S1 - 4.

The pudendal nerve is a branch of the sacral plexus (S2,3,4). It follows the course of the pudendal artery as it leaves the pelvis through the greater sciatic foramen and enters the perineum through the lesser sciatic foramen.

From here it passes anteriorly via the pudendal canal (Alcock's canal) where it divides into three main branches: the inferior rectal nerve, the dorsal nerve of the clitoris and the perineal nerve. The perineal nerve supplies the muscles of the urogenital triangle and the posterior surface of the labia majora.

The ilioinguinal nerve derived from L1 enters the anterior abdominal wall and passes down the inguinal canal into the labia majora where its branches provide cutaneous supply for both the labia and the mons pubis. Further cutaneous supply to the labia is provided by the genital branch of the genitofemoral nerve which originates from the roots of L1 - 2.

The vestibule and the perineum are also supplied by the perineal branch of the posterior femoral cutaneous nerve.

The vulva has various sensory nerve endings. Meissner's corpuscles are specialised tactile nerve endings that are particularly common in the clitoris. They consist of unmyelinated terminals of afferent nerve fibres and supporting cells that are thought to be modified Schwann cells. They are cylindrical in shape and measure about 150 microns in length. They are located immediately under the epidermis.

Pacinian corpuscles are pressure receptors found in the deeper layers of connective tissue in the labia majora and in association with erectile tissue of the clitoris. They contain the termination of myelinated nerve fibres although the terminal part itself is unmyelinated. It is surrounded by layers of flat cells that are continuous with the endoneurium. They are large ovoid structures that measure about 1mm in their long axis.

## **Histology of the vulva**

The epidermis of the vulva is a stratified squamous epithelium, developed from the ectoderm. The dermis is developed from the mesoderm. In vertical sections the epidermis undulates due to the rete ridges at the dermal epidermal junction.

Histologically the epidermis is described in 4 layers: a basal layer, a spinous or prickle cell layer which forms the majority of the epidermis. a granular layer and a horny layer.

The vestibule however is covered with a non cornified stratified squamous epithelium ie. a mucous epithelium. Hart`s line marks the border between the two types of epithelium.

Haematoxylin and Eosin stained section of the vestibule is shown in Fig 1.2.

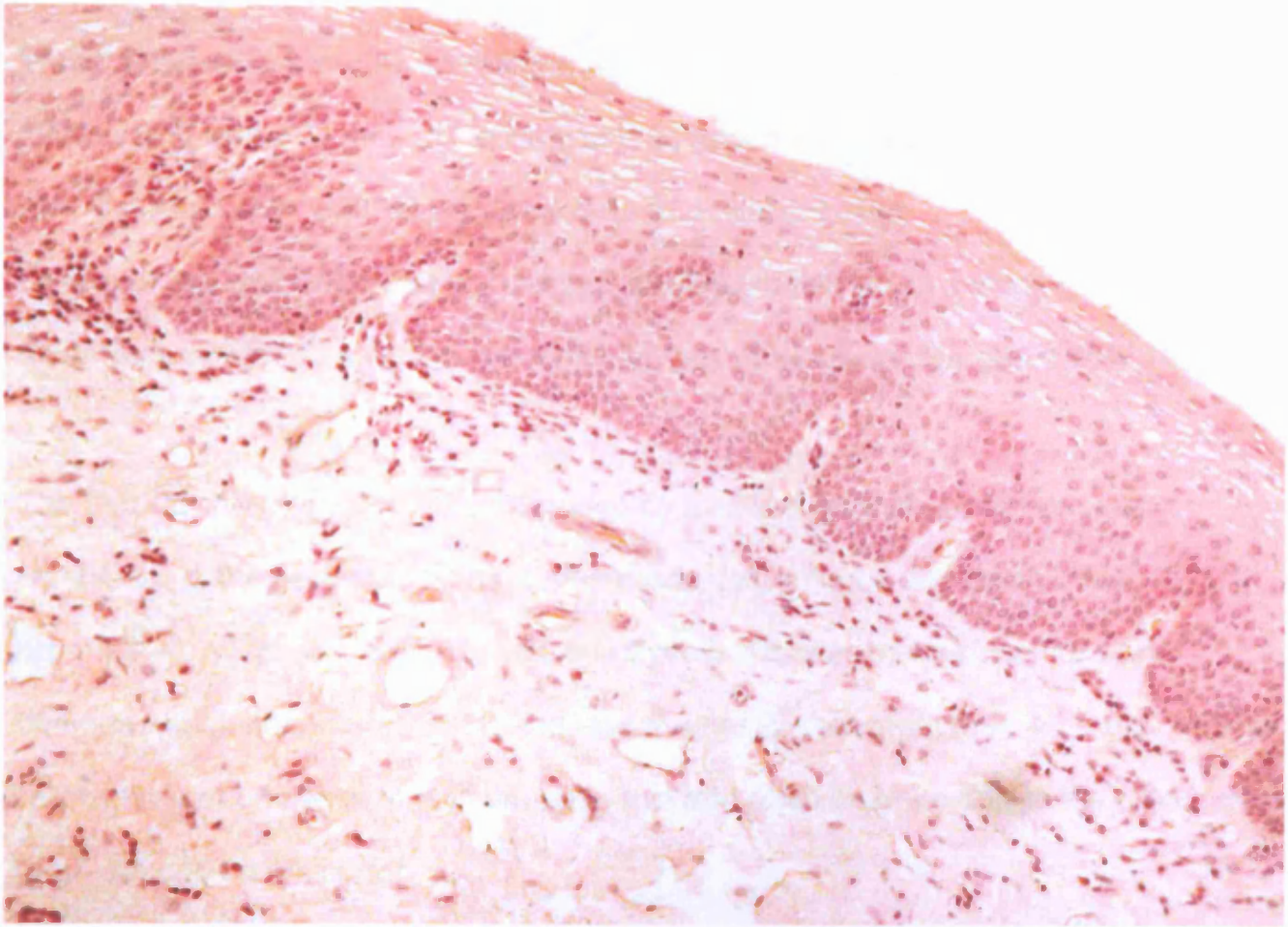
The labia majora contain a thin layer of smooth muscle (equivalent to the Dartos muscle seen in the scrotum) and a large amount of subcutaneous fat.

The outer aspects have hair follicles but the inner aspects do not.

Sweat and sebaceous glands can be seen on both surfaces

The labia minora do not contain hair follicles. In the deeper epithelial layers the cells are pigmented. Unlike the labia majora there is no adipose tissue present but fine elastic fibres are visible as are numerous sebaceous glands in the stroma.

Fig 1.2 H&E section of the vestibule



Within the vestibule, glandular structures are visible penetrating the stratified squamous epithelium. The greater vestibular glands are tubuloalveolar structures that are mucus secreting.

The clitoris is composed of erectile tissue, similar to that of the penis, covered by a thin layer of epithelium containing numerous sensory nerve endings.



### **Ultrastructure of the vulval vestibule**

The epithelium of the vulval vestibule is non keratinised and nonpigmented. Hair follicles and sweat glands are absent and its appearance is more like mucosa than that of normal skin, and stains with iodine and toluidine blue.

Layers of the epithelium are not easily indistinguishable but in a study of the ultrastructure of the vulva it is been divided into three layers.(Sargeant et al 1996)

The superficial cell layer is characterised by microridges that are not present elsewhere in the perineum. The surface cells are flattened and contain few organelles. Microvillous processes interdigitate between the surface cells and desmosomal junctions are short and less frequently seen here than in deeper layers. Nuclei are not uncommon and are spherical and often displace due to the presence of large deposits of glycogen.

The intermediate, or spinous cell layer, consists of polyhedral cells becoming increasingly flattened towards the surface. The deeper cells are rich in mitochondria and rough endoplasmic reticulum and Golgi regions are visible. The cytoplasm is characterised by the presence of free and poly-ribosomes and glycogen granules. Cytokeratin fibres form a diffuse network in the cytoplasm, leaving a clear zone around the nucleus.

The plasma membrane is highly convoluted and microvillous projections extend into the intracellular spaces between the desmosomal junctions.



The basal layer gives the basement membrane an undulating contour. Cells are characteristically round or oval and have large oval nuclei with one or more nucleoli. Mitochondria, free ribosomes and endoplasmic reticulum are numerous. As in the intermediate layer a tonofilament network around the nucleus is visible. Projections of cytoplasm extend into the dermis and lymphocytes from the underlying dermis penetrate up into the epidermis.

It is interesting to note that a significant number of inflammatory and apoptotic like cells are present in the normal vestibule. It is thought that this may correlate with high cell turnover, similar to that seen in the buccal mucosa. (Squier et al 1976)

## **Diseases of the vulva**

The classification of vulval disease has long been disputed and is regularly updated. (Ridley 1988) Fashionable terms become outmoded as our knowledge and understanding of vulval disease increases. (MacLean 1991)

The International Society for the Study of Vulvovaginal Disease (ISSVD) was founded to address these problems and to give clinicians the opportunity to meet and share knowledge.

In 1998 the ISSVD updated its classification of non neoplastic vulval disorders (Appendix 1.1) and proposed change in nomenclature relating to other pathology of the vulva was further debated at the international scientific meeting in 2003.

Vulval disease falls into three simplified categories: non neoplastic disease, neoplastic disease and vulval pain syndromes.

## **Classification of Vulval Pain**

Vulval pain is a complex and poorly understood area of vulval disease.

Vulvodynia was described in the Presidential Address of the 1985 meeting of the ISSVD as “a syndrome of unexplained vulvar pain, psychologic disability and sexual dysfunction” (Lynch 1985) and unfortunately this is probably still appropriate although our recognition, if not knowledge of the underlying mechanisms, has improved.

Vulval pain has had many names over the years. Dodson and Friedrich (1977) referred to psychosomatic vulvovaginitis and established the initial parameters of vulvodynia:

- 1) persistent symptoms of long standing duration
- 2) lack of demonstrable pathology
- 3) sexual inactivity as a direct result of symptoms
- 4) unsuccessful consultations with multiple physicians
- 5) allergy to many common vaginal preparations
- 6) reluctance to accept the suggestion of a psychophysiologic cause
- 7) emotional lability and dependence

These observations describe some of the characteristics of patients with vulvodynia but do not help in the classification of the diseases.

In the early eighties the term “burning vulva syndrome” was introduced (McKay 1984) following the report of a Task Force set up by the ISSVD.

This differentiated between vulvodynia and burning vulva syndrome, defined as vulvodynia for which no physical cause can be found, and discussed the differences of a patient who presents with pain as opposed to itch.(McKay 1985).

Although suggested that a generic term of vulval pain syndrome may encompass all patients, (Baggish et al 1995) the Latin translation of vulval pain, vulvodynia, is now universally accepted.

The term vulvodynia is defined as chronic vulval discomfort, especially that characterised by the patient's complaint of burning, stinging, irritation or rawness.

(McKay 1988) Subsets of vulvodynia at this time were classified as:

- 1) vulvar dermatoses
- 2) cyclic candidiasis
- 3) vulvar vestibulitis
- 4) squamous papillomatosis
- 5) essential vulvodynia

In 1991 the ISSVD took this further and classified vulvodynia as:

- 1) vulvar dermatoses
- 2) cyclic vulvodynia
- 3) vestibular papillomatosis
- 4) vulvar vestibulitis
- 5) essential (dysaesthetic) vulvodynia
- 6) idiopathic vulvodynia

The vulval dermatoses classically present with pruritus and not pain and will not be examined in detail in this chapter.

**Cyclic vulvodynia** relates to vulval pain that is recurrent rather than persistent and an association with the menstrual cycle can be identified. Infection such as chronic candidiasis, herpes simplex virus or bacterial vaginosis are common features.

**Vestibular papillomatosis** refers to the appearance of microscopic papillae over the surface of the vestibule. Often misdiagnosed as “microwarts”, these papillae have been subjected to a wide variety of both medical and surgical treatments, but are now regarded as a normal variant. Therefore the only treatment required is reassurance. An example of vestibular papillomatosis is shown in Fig.1.3

Fig 1.3 Vestibular papillae



**Vulval vestibulitis** refers to a specific syndrome causing vulvodynia. This will be discussed in greater depth later in this chapter.

**Essential (dysaesthetic) vulvodynia** typically occurs in older women.

It is an intense burning of the vulva with no physical signs. The burning is persistent and unrelenting but pruritus is absent. This pain is often compared to glossodynia or

post herpetic neuralgia and as a consequence of this the same treatment protocols have been applied. (Watson et al 1982) Often low dose tricyclic antidepressants will provide total relief, but sometimes much larger doses are necessary. (McKay 1993)

**Idiopathic vulvodynia** incorporates unusual cases of vulval pain that are not classified by the major subgroups.

Infective conditions of the vulva are not covered by these classifications but are common causes of vulval pain as opposed to itch. (Reid et al 1995) These are not often seen in the vulval clinic as they tend to be treated elsewhere, most commonly in the genito-urinary medicine clinic.

Although many of these infections are thought to be vaginal involvement of the vulva in many cases is inevitable. This includes conditions such as genital herpes, trichomoniasis, candidiasis, herpes zoster, chancroid and amoebiasis.

As the vulva is a hair bearing area, folliculitis and subsequent abscess formation may occur, but these can normally be treated simply with oral antibiotics.

Vulvodynia continued to be debated and there were proposals to no longer use this term.

At the 2003 ISSVD meeting in Brazil, it was voted to return to use of the well accepted term of vulvodynia, with a slightly modified definition, of "Vulvar discomfort, most often described as burning pain, occurring in the absence of relevant visible findings or a specific, clinically identifiable neurologic disorder."

The classification of vulvodynia, based on the site of the pain was also introduced.

This is the most recent classification of vulval pain, (2003):

A. Vulvar pain related to a specific disorder

1. Infectious (e.g.candidiasis, herpes)
2. Inflammatory (e.g. lichen planus, immunobullous disorders)
3. Neoplastic (e.g. Paget's disease, squamous cell carcinoma)
4. Neurologic (e.g. herpes neuralgia, spinal nerve compression)

B. Vulvodynia

5. Generalized

- a) Provoked (sexual, nonsexual, or both)
- b) Unprovoked
- c) Mixed (provoked and unprovoked)

6. Localized (vestibulodynia, clitorodynia, hemivulvodynia)

- a) Provoked (sexual, nonsexual, or both)
- b) Unprovoked
- c) Mixed (provoked and unprovoked)

# **Vulval Vestibulitis Syndrome**

## **History**

Vulval pain is not a condition purely of the modern age. Although the term vulval vestibulitis syndrome (VVS) has only been in use for over a decade, the clinical entity we currently call vestibulitis has been making appearances in the literature for over a century.

The earliest record appears to be in 1889 when Skene recognised hyperaesthesia or excessive tenderness of the vulva without pruritus and the absence of external manifestations. However during examination he said “when the examining finger comes into contact with the hyperaesthetic part, the patient complains of pain which is sometimes so great as occasion her to cry out.”

In 1891 in their text Thomas and Munde commented on hyperaesthesia of the vulva and described it as “ a malady which consists in an excessive sensibility of the nerves supplying the mucous membrane.”

No further comment is made until 1928 when Kelly described “exquisitely sensitive deep red spots in the mucosa of the hymenal ring as a fruitful source of dyspareunia.” He commented that these areas were so tender as to make a pelvic examination essentially impossible.

The vestibular glands were described in detail in a variety of anatomy texts over the next forty years but no connection was made with the previous clinical descriptions.

(Hunt 1942, Dickinson 1949, Friedrich 1973)



In 1976 a series of 30 cases was presented of women who had red spots localised to the posterior vestibule and dyspareunia as a major symptom. Histologically there was an abundance of plasma cells but the authors did not associate this with the glandular element of the vestibule (Pelisse and Hewitt 1976).

Plasma cell balanitis of Zoon was already recognised and in a review of the literature "vulvitis circumscripta plasmacellularis" was suggested as a cause of vulval pain. However this also was not associated with the vestibular glands (Davis 1983).

In 1983 Friedrich published his description of the vestibule and introduced the term vestibular adenitis. He described the vulval vestibule as an active and reactive area and vestibular adenitis as a condition when the minor vestibular glands and sebaceous glands become inflamed which produces a painful erythematous vestibule. When pressure is applied to these spots pain is provoked. He suggested that multiple glands become involved and the spots coalesce into a diffuse, tender erythema that may extend to involve a considerable part of the vestibule. He proposed that in chronic cases of vestibular adenitis that mucus glandular elements have been replaced by squamous metaplasia and only the plasma cell rich infiltrate remains. Because of this squamous metaplasia he emphasised the importance of not mistaking the condition for carcinoma in situ (Friedrich 1983).

This view was reiterated when he combined data with Woodruff and commented that the diagnosis is made due to the lack of normal stratification in the epithelium. They described vestibular adenitis as a newly recognised syndrome of severe dyspareunia caused by inflammation of the minor vestibular glands (Woodruff and Friedrich 1985).

At the same time as the term vestibular adenitis was introduced Woodruff and Parmley (1983) described a condition of tiny erythematous foci immediately lateral to, and circumferentially involving the hymenal ring. The hymenal ring was constricted and rigid with associated oedema and these foci were tender on palpation. No other physical abnormalities were seen and many of the patients described had been labelled as having psychiatric problems. The authors suspected that these signs and symptoms were caused by a chronic infection of the minor vestibular glands.

The term focal vulvitis was then introduced (Peckham et al 1985). This was defined as "a unique syndrome characterised by severe and persistent dyspareunia and presence of one to eleven minute, exquisitely tender areas of focal inflammation or ulceration on the mucosa of the vestibule." It was observed that three quarters of the lesions were around the major (Bartholins) vestibular glands, or between them posteriorly.

A case definition for focal vulvitis was set out with criteria including vulval pain, dyspareunia or pain with tampon insertion associated with:

1) one or more minute exquisitely sensitive focal inflammatory lesion located in the vestibule.

2) no identifiable cause eg. herpes, bacterial or candidal vaginosis or pemphigoid.

Peckham et al described mucosal changes (1985). 22% of the cohort had minute discrete ulcers in the mucosa, some patients had superficial denuded mucosa or appeared split and tiny indolent ulcers were deep within the crypts. It was thought that these were secondary to inflammation but not necessarily infection.

The review of their histology did not reveal excessive plasma cells leading them to state that they had excluded the possibility that these women had Zoon's vulvitis, but had a separate condition, focal vulvitis.

A year later minor vestibular gland syndrome was introduced (Marinoff and Turner 1986). This was described as a specific clinical entity found in young women with a previous history of vaginal candidiasis.

The criteria for diagnosis included introital dyspareunia, absence of active infection, erythema around the orifices of the minor vestibular glands and exquisite tenderness over these gland openings.

The aetiology of this was unrecognised but the authors concluded that it was unlikely that a delayed hypersensitivity reaction to candida or an irritant or allergic reaction to vehicles in vaginal cream were responsible.

In 1987 Friedrich argued that the terms previously used should no longer apply.

Having reviewed the histology he concluded that since secretory acini and gland ducts were not intrinsically involved by inflammation and the presence of glandular elements in the involved foci was not constant that adenitis was not an appropriate description. He also suggested that the term focal vulvitis was too broad and could apply to any part of the vulva involved in local irritation. Instead he proposed the term Vulval Vestibulitis Syndrome (VVS) and laid out strict criteria to be fulfilled before the diagnosis could be made. These criteria are now known as Friedrich's triad.

VVS is a constellation of histological, subjective and objective criteria:

- 1) severe pain on vestibular touch or attempted vaginal entry.
- 2) tenderness to pressure localised within the vestibule.
- 3) physical findings confined to vestibular erythema of varying degrees.

This term and definition is in general use today and was approved by the ISSVD, who agreed, "vulval vestibulitis syndrome does not include symptoms associated with acute inflammatory conditions or with immediate post operative changes." They have suggested that the specialised epithelium of the vestibule and minor vestibular glands may make this area particularly sensitive to morphological changes that may influence the development of vulvodynia.

The terminology is still a matter of great debate and the latest suggestion from the ISSVD is "localized provoked vulvar dysesthesia" (2001), although VVS still appears to remain the common term of use.

Since Friedrich's definition it has been postulated that there may be subsets of vulval vestibulitis.

In patients whose dyspareunia invariably involved the greater vestibular gland duct with or without sensitivity extending to adjacent sites may make up one subgroup of vulval vestibulitis (Michelwitz et al 1989).

Vestibulodynia is proposed as a subset of vestibulitis in women who fulfil Friedrich's triad but who also have a persistent pain of vestibular origin (Bornstein et al 1997).

### **Diagnosis of vulval vestibulitis syndrome**

The diagnosis of this condition can be made following the taking of a thorough history and after detailed physical examination. Classically the patient is a young woman who previously has had no vulval symptoms. She often presents to a specialist vulval clinic after many months or years of symptoms. It is not uncommon for the clinic to be among the last of many consultations, having seen many clinicians in a variety of departments. Often she has been misdiagnosed, with the most common diagnosis being chronic candidiasis. It is not unusual that she has been told that there is nothing physically wrong and that her condition is psychosomatic, and may have been offered psychosexual counselling.

Her symptoms can be sufficiently severe that it has had a major impact on her lifestyle and may well have contributed to the breakdown of relationships.

She is often angry, cynical and disillusioned with the medical profession and it is important to build a good relationship with these patients quickly, although this may not always be easy.

The typical story is of a perfectly normal sexual relationship until the onset of symptoms. Normally she can identify the point at which the symptoms started and often the onset was extremely quick. She describes pain at the point of penetration during intercourse. Often if intercourse is possible she describes that the initial pain wears off and she does not complain of deep dyspareunia. Some patients also complain of an intense vulval burning following intercourse that may last for hours, if not days.

As a result of these problems intercourse has become less frequent, or impossible. If specifically asked about tampon use, often patients will find this uncomfortable and many have abandoned their use. In severe cases certain items of clothing, such as tight jeans may have become uncomfortable to wear. In addition certain other activities, such as horse riding may exacerbate the symptoms.

Many patients have tried multiple treatments, both conventional and the more unusual, with little success. Examination prior to referral has usually been reported as unremarkable. This is where the specialist facilities of a vulval clinic can enable the examining doctor to make a diagnosis.

Diagnosis of VVS with the naked eye in a routine clinic on a normal couch is difficult, but appropriate lighting and adequate exposure on a colposcopy couch or chair makes the process much easier, and if closed circuit television is attached to the colposcope enables to patient to see problem areas.

Colposcopy of the vulva is now well recognised (Byrne et al 1989). However, with vulval pain syndromes, often physical findings are minimal or non existent (Reid et al 1988). Equally it has been recognised that signs such as vestibular erythema can be identified in healthy women with no vulval symptoms (van Beurden et al 1997).

Examination of the vulva should be conducted in a private clinical area, ideally a separate room and not a curtained off area. Adequate lighting should be available. Optimum examination is in the lithotomy position and a tilting chair is ideal to achieve this. The whole vulva should be carefully examined, including the groin and perianal areas, both with the naked eye and with the aid of the colposcope.

The use of KY jelly may be of assistance in minimising the reflection of light in hair bearing areas (Reid and MacLean 1995).

Other vulval lesions, as previously discussed may be visualised with the use of acetic acid or toluidine blue, but this is not necessary in the diagnosis of vulval vestibulitis syndrome.

In VVS the labia will have normal appearance. Physical appearance of the vestibule may be variable. The most common colposcopic finding is enlargement and erythema of the orifices of both the paraurethral and posterior vestibular glands, although each

may be seen independently. Erythema of the vestibule is one of the defining criteria, but as suggested in the original definition the degree of erythema may be variable.

In a trial investigating the reliability of diagnosis and evaluation of the current diagnostic criteria of VVS it was noted that “inter rater” agreement and test – retest reliability for the presence or absence of erythema was poor, suggesting it may not be a significant contribution to diagnostic decision making (Bergeron et al 2001). Support for this is provided by Friedman (1995) who found normal controls not significantly different from VVS women in terms of presence of erythematous lesions.

Touching of the vestibular glands will produce exquisite tenderness, evoking the pain experienced at attempted intercourse. Traditionally this has been referred to as “Q tip tenderness” as a cotton bud is rolled over the entrance to these glands (Goetsch 1991). Bergeron et al (2001) found that VVS can be reliably diagnosed in women with dyspareunia, and the pain can be rated and described in a consistent fashion by these women. However, pain assessment at examination between different gynaecologists varied, as a result of different degrees of pressure applied.

It has been found that a vulval algesiometer provides a more accurate assessment of this tenderness. (Eva et al 1999) The use of the vulval algesiometer will be discussed in greater detail in chapter 2.

A full set of swabs should be taken to exclude infective causes of acute vulvovaginitis, eg. candida, chlamydia, gonorrhoea, trichomonas, bacterial vaginosis. If the history of entry dyspareunia, physical findings of erythema around the vestibular glands and tenderness when pressure is applied to these openings are present, then the diagnosis of vulval vestibulitis syndrome can be made.



## **Pathology of vulval vestibulitis syndrome**

The first attempt to characterise the pathology of VVS was in a series of 41 patients (Pyka et al 1988). A wide variety of pathological investigations were performed on the tissue obtained from patients who had undergone surgical excision of the vestibule. Sections stained by haematoxylin and eosin were examined for histopathological features. The predominant feature was a mixed chronic inflammatory infiltrate. 75% of sections had lymphocytes present and 75% had plasma cells, although they were never the predominant cells. Histiocytes were present in 30% of the samples and polymorphonuclear leucocytes in 25%. Eosinophils were extremely rare.

Unlike previous clinical descriptions, there was never any evidence of ulceration or necrosis. No evidence of atypia of any cell was present. The inflammatory infiltrate was often perivascular and in the majority of the specimens was categorised as mild to moderate. Inflammation in the minor vestibular glands was periglandular and was never seen to cause an adenitis by involving the gland itself, thus agreeing with Freidrich`s argument that vestibular adenitis was no longer an accurate description of this condition.

All minor vestibular glands showed some degree of metaplasia with some having almost been replaced by squamous metaplasia.

The authors described vestibular clefts, which had been formed by the coalescence of clusters of adjacent glands, which had undergone squamous metaplasia.

These clefts almost took on the appearance of the surrounding vestibular epithelium.

The lymphocyte population that was so prevalent, was almost exclusively

T lymphocytes. B lymphocytes were absent in all but one of the specimens.

Staining with toluidine blue for mast cells proved positive in less of the study population than in the control group. Immunofluorescence staining was performed for Ig G, Ig A, Ig M, complement and fibrin. All results were non-specific. Organisms were looked for using silver methanamine stain for fungus, Gram staining, Brown – Brenn stain, Acid fast stain and Dieterle silver stain specific for spirochetes but all proved negative.

In summary the study could not define a characteristic histopathology for vulval vestibulitis, and only a non-specific chronic inflammatory infiltrate had been revealed.

In a review of 114 non neoplastic vulval biopsies only 11 patients had vestibulitis and the authors concluded from this that the clinical syndrome of vestibulitis did not have a specific histological picture. The biopsies showed non-specific inflammation, mild hyperplasia or were normal. (O'Keefe et al 1995)

A further series of 24 patients was published. (Furlonge et al 1991) They found the most common histopathological pattern was epithelial hyperplasia accompanied by an acute or chronic inflammatory cell infiltrate in the lamina propria.

Specifically they found koilocytosis in 2, neutrophilic vasculitis in 1 and acute inflammation of a sebaceous gland in 2 specimens. All stains for microorganisms were negative. The authors suggested that due to the non-specific pattern of the pathology, VVS may be the end point of a number of pathological processes.

A different argument was put forward following publication of a series of 16 patients. (Chaim et al 1996) Following staining with Giemsa, large numbers of mast cells were found in the specimens of patients with a diagnosis of vulval vestibulitis. Mast cells were not seen in the control tissue. Apart from this finding there was not a great difference compared to the other studies, showing chronic inflammation, parakeratosis, hyperkeratosis, oedema, koilocytosis and acanthosis. The authors suggested that mast cells play a role in the aetiology of the symptoms of VVS and made comparisons with the role the mast cells are known to have in interstitial cystitis. They suggested that vestibular pain and inflammation is mediated by a similar method, as in interstitial cystitis, where prostaglandins and histamine are released by mast cells "recruited" to a local site by increased numbers of unmyelinated afferent and sympathetic efferent nerve fibres. These fibres exert a tropic effect on the mast cell density. This would also explain the erythema, caused by vasoactive mediators found in mast cells. These findings however conflict with those of Pyka et al (1988) where staining for mast cells was not as marked.

More recently, in a series of 12 patients, (Chadha et al 1998) no great difference was found compared to previous studies. This study showed a chronic inflammatory infiltrate in the dermis that extended into the epidermis in 66% of the samples. Predominant cells again were lymphocytes and immunophenotyping of the lymphocytic infiltrate showed mainly T cells with a small number of B cells. The T helper/ T suppresser ratio was normal. Other cells identified were plasma cells, mast cells and occasionally monocytes.

The minor vestibular glands were associated with a periglandular inflammatory infiltrate, which, as previously recognised, did not invade the gland itself. Squamous metaplasia was present in a third of the specimens, which was a lower rate than had previously been quoted. Reactive changes were seen in the mucosal squamous epithelium. Epithelial hyperplasia was seen in 83%, hyperkeratosis and parakeratosis in 50% and no ulceration was seen in any of the samples. In situ hybridisation for human papilloma virus subtypes 6, 11, 16 and 18 were negative but the association between HPV infection and VVS will be addressed in more detail later in this chapter.

In summary, current literature is ambiguous and there are no defining pathological features to sustain a diagnosis. The changes seen in the histopathology of vulval vestibulitis syndrome remain that of non-specific chronic inflammation with no characteristics features.

The challenge is to find more useful diagnostic parameters to allow better reliability of diagnosis.

## **Epidemiology of vulval vestibulitis syndrome**

Various studies have been conducted in an attempt to define the population that this condition affects. The majority have compared characteristics of their study populations to a group of normal controls to see whether any dramatic difference is apparent. Unfortunately this does not appear to be the case, although certain patterns have developed.

The most obvious characteristic is that VVS seems to mainly affect the Caucasian population. Although this could be expected in a Scandinavian population (Sjoberg et al 1997) it is more unusual in North American studies that have a multicultural general population. (Goetsch 1991, Peckham et al 1985, Mann et al 1992, Schover et al 1992) Therefore there was a need for a London study where there is a varied multiethnic population.

Classically this is a condition of young premenopausal women normally presenting in their twenties or thirties, although the symptoms of the condition may have occurred some time before presentation.

The length of symptom duration reflects the fact that a visit to specialist may be at the end of a long search via many clinicians for answers. Usually duration of symptoms is at least a year, but median duration varies from 23 months (Mann et al 1992) to 8.5 years. (Goetsch 1991) The majority of patients in all studies are nulliparous.

Whether this is reflection of the fact the condition affects younger women, or whether the impact of their symptoms is enough to seriously impair their ability to tolerate penetrative intercourse is unclear. Some authors class post-partum vestibulitis as a subset of VVS (Goetsch 1991) but there is little evidence elsewhere to support this.

Age of first intercourse appears in concordance with the general population although one study found an increase rate of vestibulitis (relative risk 3.3) in the group of their cohort who had first intercourse under the age of 16. (Bazin et al 1994)

Number of lifetime partners varies minimally between studies (median 3.6 (Sjoberg et al 1997) and 5 (Peckham et al 1985), but this is not a syndrome of promiscuous women.

Contraceptive use analysis shows that the oral contraceptive pill (OCP) is the most commonly used form of contraception, and Bazin et al 1994 showed an increased relative risk of developing VVS in women who had used the OCP before the age of 17 and suggested that it's use may be involved in the aetiology of VVS. However it is used by less than half of the women in most cohorts.

In general there are few characteristics of women who have VVS that set them apart from any other gynaecological population.

In conclusion, from the studies looking at epidemiology, we can surmise that this is a condition that affects mainly white women in their twenties and thirties, who are likely not to have children.

## **Aetiology of Vulval Vestibulitis Syndrome**

Since the discovery of VVS as a clinical entity, regardless of name, the search began to discover aetiological agents that may explain the unusual course of the condition. Various avenues have been pursued and publications have produced a range of possibilities.

Attempts have been made to sub classify VVS as different pathologies in order to explain aetiology further. Acute vulval vestibulitis syndrome could be caused by any infective cause of vulvovaginitis, or by contact irritants such as soaps, latex, spermicide and douches. Removal of these precipitating factors could resolve the condition. Chronic VVS could be caused by chemical therapeutic agents such as antiseptics or applications of local antifungals or antibiotics. Destructive therapeutic agents such as cryosurgery, podophyllin or tri-chloroacetic acid could precipitate symptoms, as could drug reactions (Marinoff and Turner 1991).

Another suggested subdivision is that VVS is either primary or secondary (Goetsch 1991). This author argued that the syndrome exhibits bimodal occurrence that is either primary, where the patient has never been able to have painfree intercourse, or acquired later in life after a precipitating factor, where previous painfree intercourse had been possible.

Characteristics of primary VVS are patients that give a history of worse pain compared to those with secondary VVS and there is a strongly positive family history, affecting the patient's immediate female relatives. An increased proportion of nulliparous women are found in this group.

Secondary VVS is often post partum. There is a negative association between sisters and specific causes are often implicated. e.g. group B Streptococcus, although this is a vaginal commensal, carried by 14% of the pregnant population, and may not be significant.

One study found 44% of their cohort experienced symptoms with first intercourse, and therefore were classified as primary VVS (Bazin et al 1994).

They postulated that VVS was exacerbated by loss of protective vaginal mucus. They hypothesised that primary VVS was due to a physiological malfunction of the minor vestibular glands producing inadequate amounts, or impaired quality of mucus and that secondary VVS was when the mucus production was affected by an acquired cause. An example of such was when the quality and quantity of mucus is modified by hormones.

Argument against the idea of primary and secondary VVS is provided in a study where only 6 of 50 women experienced symptoms with first intercourse and 1 with first use of tampons. The remaining 43 had normal relationship and lifestyle prior to the commencement of their symptoms (Peckham et al 1985).



### **Infective causes**

Probably the most extensively investigated possible aetiological factor is human papilloma virus (HPV). Histopathological studies revealed the presence of koilocytosis, previously considered to be an indicator of the presence of HPV. The quoted incidence of koilocytosis in patients with VVS is variable, and ranges between 16 and 71%. Obviously a lot of women find the diagnosis of HPV, and the stigma of genital warts distressing, and so is important in these patients, who are already distressed, not to label them with an additional and unnecessary diagnosis. It is not logical that HPV could cause VVS, as HPV infection alone is normally painless, and even vulval warts do not usually produce the pattern of vulval pain seen in VVS.

The theory linking VVS and HPV was first proposed in a small study of just 7 women (Turner and Marinoff 1988). These authors looked for HPV DNA using Southern blot techniques and found evidence of the virus in all of their samples. However they were unable to subtype any of the HPV for the common subtypes seen in genital infection, (types 11, 16, 18 and 31) with the exception of one sample that was positive for HPV type 6. Histopathological examination of these specimens did not show koilocytosis in any of the samples. They proposed that HPV infection is one of the causes of vulvodynia and VVS. They thought that the inflammatory response, in the form of a lymphohistiocytic infiltrate around the superficial capillaries, may be the beginnings of the body's attempt to rid itself of virus and the syndrome was perhaps due to a problem with the local immune response.

They concluded that the treatment of HPV infection, for example by interferon may cure introital dyspareunia.

A further study of 13 patients (Umpierre et al 1991) appeared to concur with this. HPV was searched for using polymerase chain reaction (PCR) and dot blot hybridisation with a non-specific primer. They found HPV in 85% of the samples. 50% were types 16 and 18, the remainder were unidentified. They looked at patient response to alpha interferon, but found it only to be effective in half of the HPV positive patients. They concluded that although HPV appeared to have a role in the aetiology of VVS, HPV detection did not help in predicting which patients would respond to interferon.

Goetsch in the same year, in a much larger study did not find significant evidence to support this. Studies since then have contradicted the initial evidence.

A study of 31 women (Wilkinson et al 1993) used a PCR probe specific for HPV types 6, 11, 16 and 18. They found koilocytosis in 5 patients and only 3 of these produced a positive result for HPV (types 11 and 16). The prevalence rate of 10% was similar to that seen in their control population. They argued that this difference compared to previous rates may be due to the fact that they did not probe for all known HPV types, but only for the most common ones seen in genital infection.

However two studies published shortly after this study produced similar results, both using PCR technology. One (Bazin et al 1994) quoted a prevalence of only 5.3% and

the other failed to identify HPV in any of the 11 patients studied, including one who demonstrated koilocytosis (Bergeron et al 1994).

Only one study has looked specifically for other viral aetiology (Bornstein et al 1995). They looked at 86 women using PCR on frozen sections to investigate the presence of HPV, herpes simplex virus (HSV) and cytomegalovirus (CMV). No evidence of HSV or CMV was found in any of the samples. The prevalence of HPV was 54% although was not typed for 6,11, 16, 18 or 33. They concluded that HPV is found in more than half of cases of VVS.

It has been proposed that the variability in the rate of HPV infection could be due to the different methods used. DNA may be lost due to subtle differences in the extraction, and the search for specific types may bias results. With such conflicting reports, the evidence that HPV infection may be part of the aetiology of VVS is inconclusive.

The emphasis remained on an infective cause being the major aetiological factor, with *Candida* species being the most likely culprit. In his review of focal vulvitis (Peckham et al 1985) *Candida* was isolated in 67% of the cohort. There was no evidence of gonorrhoea, chlamydia or herpes simplex infection in any of the samples. 9% of the cohort had evidence of non-specific urethritis. Half of the group cultured positive for ureaplasma, however this is comparable with the rate found in normal healthy women and is probably not significant.

The focus remained on candida and the theory was proposed that in some patients the antigens of *Candida albicans* are cross reactive with certain vulvovaginal tissue antigens. They suggested that eventually an effective immune response against *Candida albicans* is aborted. This leads to a local inflammatory “response against self” after repeated infections, which may be triggered by hormonal influences or stress (Ashman and Ott 1989).

We now know that although the majority of patients with VVS claim to have recurrent candida over the years this probably just reflects the high rate of misdiagnosis in this condition and is not the cause. Therefore this theory does not seem likely.

Two studies in the same year did not find a higher rate of infective agents although it was proposed that recurrent bacterial vaginosis could contribute to symptoms as alkaline pH can be as irritant as acidic pH (Marinoff and Turner 1991).

The other study found that Group B streptococcus was the only infective agent to directly cause or worsen symptoms, although the prevalence was low (Goetsch 1991).

Two conflicting studies were then published, one quoting *Candida albicans* prevalence of 80% (Mann et al 1992) and the other a much lower rate of 8.8% (Bazin et al 1994).

The Canadian group took detailed microbiological swabs and again the presence of gonorrhoea, chlamydia, trichomonas and mycoplasma were not found. Bacterial vaginosis was present in 14% and ureaplasma in 17% of the cohort.

With the exception of *Candida* species, the evidence is fairly conclusive that there is no link between bacterial or fungal agents and VVS.

It is unlikely that *Candida* is the cause of VVS, as most patients have been repeatedly tested and treated, even with negative results, for yeast infection prior to presentation in a specialist clinic, with no effect on their symptoms.

However it may be possible that fungal infection may act as a precipitating event to start the pathological process of VVS.

### **Hormonal factors**

Little attention has been paid to the role of hormones in VVS. Several studies have alluded to possible links with situations affecting hormonal status, such as pregnancy and contraception, but no published work has concentrated on this aspect.

Goetsch (1991) stated 21% of her cohort of patients developed symptoms post partum, although the possibility that VVS was related to perineal trauma at childbirth cannot be supported, as she had a greater proportion of nulliparous than multiparous women in her cohort.

Another study suggested that post partum VVS was secondary to the hypo-oestrogenised state of lactation, but specific comment was not made as to the proportion of women who had delivered who were breastfeeding. It was also proposed that sexual dysfunction could be due to fatigue or fear of dyspareunia (Schover et al 1992). However, women with post partum VVS had symptoms that

continued for many years, even once they had stopped lactating and the hormonal balance had been restored. The authors also noted that in their cohort many women reported exacerbations of pain in the luteal phase of the cycle and during menstruation, and suggested that perhaps this was an indicator of a hormonal effect on the condition.

Only two studies have specifically postulated a role for oestrogen in the pathophysiology of VVS and these were concluded from information on contraception, specifically the oral contraceptive pill (OCP).

Bazin et al (1994) found that women who had ever taken the OCP had a relative risk of developing VVS of 5.8 (95% confidence interval 0.7-47.0) when compared to a control population. Those who were currently using the OCP as contraception had a relative risk of 6.8 (95% confidence interval 0.8-55.6) and those who had used the OCP early in life, i.e. below the age of 17 had a relative risk of developing VVS of 11 (95% confidence interval 1.3-97.1). Length of use did not appear to be a determining factor.

They also found that an early menarche nearly doubled the risk of developing VVS, and increasing parity decreased the risk. From this they concluded that factors affecting the condition could be hormonal, and events occurring early in the woman's reproductive life are important determinants of her developing VVS. They proposed a possible mechanism as alterations in mucus, which has an irritant effect. It is known that oral contraceptives have an effect on glandular components and mucus secretion

and this study argued that this could offer an explanation for the apparent connection with the OCP.

The other study to look at OCP, performed in Sweden, claimed that the argument proposed by the Canadian study, is contradictory, as a recognised treatment is vestibulectomy where the mucus producing glands are removed and the patient improves.

Their data found no difference in the parity of VVS patients compared with controls, but found the VVS patients had used the OCP for a significantly longer period of time (Sjoberg et al 1997). The same group had previously shown that OCP downregulates oestrogen receptors (Sjoberg et al 1989).

Their suggested mechanism was that if the OCP was started early, or used for a long period of time then the oestrogen receptors would become downregulated in the vestibule, which would lead to the epithelium becoming thin and fragile.

For those women with VVS not on the OCP, they suggested that even without the OCP they may have few oestrogen receptors, leading to a thin epithelium which is irritated by the acidic vaginal environment.

In a presentation to the ISSVD in 1999 Willems presented results of medical treatment and found good response to topical oestrogen cream, but no mechanism for action was proposed.

Many of the above authors have recommended that further work be done specifically on possible links between hormonal status and VVS.

## **Psychological factors**

Patients with vulval pain are often labelled as having a psychosomatic disorder, although often this is more likely to be due to the ignorance of the clinician than the underlying disease process. Certainly one route of referral to a specialist vulval clinic is via a psychosexual counsellor. Certainly psychological factors play a role in vulval disease but it is not obvious whether these problems are a result of the vulval pain or a cause of it.

In VVS there are identifiable physical features and therefore the latter is unlikely.

Studies looking at vulval pain in general found that vulvodynia patients are more psychologically distressed compared with women with other vulval pathologies. Essential vulvodynia causes more distress than vulval pain of an identifiable physical cause (Stewart et al 1994).

Factors as to why this is the case are inconclusive. Suggestion has been made that trauma could play a role in the development of vulval pain but no difference was found in the incidence of childhood physical or sexual abuse in women with vulvodynia compared to women with vulval symptoms from known pathology (Edwards et al 1997).

Relatively few studies have specifically looked in detail at the psychology of patients with VVS. In the first publication to address this problem, Schover et al (1992) found 51% of their cohort complained of other non genital pain syndromes. They also found



a depression rate of 36% and somatisation of 42%. In their group, vulval pain occurred during periods of stress and this led to other problems such as vaginismus. They presented the hypothesis of a complex interactive process that women are vulnerable physiologically due to an unknown local or genetic factor, and vulnerable psychologically if they tend to react to stress with somatic symptoms, especially chronic pain. This leads to decreased sexual desire and arousal, which may contribute to a degree of vaginismus. Further episodes of repeated trauma of coitus in an unaroused state then leads to vulval inflammation and further psychological distress.

Another study linked VVS to increased somatisation and shyness (van Lankveld et al 1996). They found no difference in psychological distress, marital dissatisfaction or risk of psychopathology compared to a control population.

This was the only study that analysed the behaviour of the partners of women with VVS. They concluded that both the women with VVS and their partners were psychologically healthy, although VVS may be associated with a “situationally defined sexual dysfunction” for the woman. This was due to the fact that the women had increased problems with arousal when their partners were present but less problems with masturbation than the control population. This is understandable given the stresses that sexual dysfunction of any sort places on a relationship.

Their final conclusion was that VVS is part of a circular process in which physical condition and sexual dysfunction interact.

Parallelism between VVS and somatisation disorders has been suggested (Jantos and White 1997). They highlighted the psychogenic problems brought about by “symptoms not caused by organic factors”. They made a comparison with premenstrual syndrome, of which they reported a prevalence of 85% in their cohort of women with VVS. Stressing the lack of organic factors the authors suggested that it is, by default, a psychogenic problem. This study emphasised that the persistence of pain will have a debilitating effect on the patients’ psychological well being.

Chronic pain is frequently associated with depression and estimation of the prevalence of mood disorder in chronic pain patients varies considerably, reflecting both the short-comings of the measures and diagnostic procedures used, and variations in the populations studied.

The authors illustrated this by showing that the cohort self reported a depression rate of 69%, but using the Beck Depression Inventory they classified 91% as having moderate to severe depression and 57% having expressed suicidal ideation or intent. However it was not possible to exclude the premenstrual syndrome component of depression from VVS patients. They did not find any evidence of phobic or hypochondriacal behaviour.

It is very easy to classify the difficult patient as mad, especially when solid physical features are difficult to find. It is not surprising, given the length of duration of most women’s symptoms in VVS, that they become obsessed with their problems, considering the enormous impact that this condition has on their relationships and lifestyles. Certainly there is a psychological component to VVS but this is more likely

to be a sequelae of the frustration brought about by the lack of diagnosis or successful treatment. Sometimes purely agreeing that there is a physical problem is enough to stop these women questioning their sanity.

### **Other aetiological factors**

Several other possibilities have been suggested, the two most commonly quoted are the relationship between VVS and diet and that with interstitial cystitis.

A case report first suggested that there was a link with oxalate and VVS, in which a patient was presented with fluctuating hyperoxaluria and increased urinary pH. The authors hypothesised that oxalate crystals excreted in the urine are irritant to the vulval epithelium and could give rise to the physical findings. The patient was treated with calcium citrate and a low oxalate diet and she improved (Solomons et al 1991).

This was an isolated case and further studies have not supported this evidence.

Poole and Munday (1999) investigated 31 patients with VVS for abnormal oxalate intake or excretion. None of these women were found to have increased levels of urinary oxalate. One third of the cohort improved with a low oxalate diet without calcium citrate but they could find no evidence to suggest that it was the reduction of oxalate content that altered symptoms.

A further study also showed that there was no difference in the 24 hour excretion of oxalate between women with VVS and healthy controls, in which the authors concluded that although urinary oxalate may be a non-specific irritant that aggravates vulvodynia, its role as an instigator is doubtful (Baggish et al 1997).

A link between interstitial cystitis and focal vulvitis was first proposed in 1990.

(McCormack) This was reiterated as a link between VVS and interstitial cystitis based on the evidence that both the vestibule and the bladder are of urogenital sinus origin. (Fitzpatrick et al 1993) Neither condition has specific histopathological features and non-specific inflammation and immunoglobulin deposition are common to both. The subtle differences in pathological findings are explained by the different architectures of the two structures. The authors suggested that there was a psychological component to both conditions and raised the possibility of an allergic factor that may increase the epithelial permeability to allergens. The authors presented three patients with concurrent VVS and interstitial cystitis and suggested that this may be a generalised disorder of urogenital sinus derived epithelium.

A later study tried to show parallel pathologies between the two conditions (Stewart and Berger 1997). It is known that interstitial cystitis shows epithelial defects and absent or greatly decreased mucous layer and decreased acid mucopolysaccharide content when stained with periodic acid - Schiff/colloidal iron staining with van Gieson counterstaining and immunofluoresence (Gillespie et al 1990). In addition both conditions are associated with increased angiogenesis, which is visible in the vestibule, and at cystoscopic examination of bladder mucosa.

A later paper by the same author suggested that abnormal arylalkylamine metabolism in interstitial cystitis produces pathological changes via both blood and urine. This ischaemia, together with abnormal urinary metabolites, result in the loss of the protective mucous membrane in the bladder, allowing urine to contact exposed nerves and therefore causing pain (Gillespie 1993).

Stewart and Berger (1997) hypothesised that a similar process affected the vulval epithelium in VVS. However their results showed no defects in the epithelium of VVS patients, although there was some complement deposition along the dermo-epithelial junction, and perivascular IgM. They recommended further investigation into the possible role of complement in these two conditions.

A further investigation was performed to examine the inter-relationship between VVS and urethral syndrome by looking at urethral pressure variability (Foster et al 1993). They found that VVS was associated with an increase in urethral pressure variability compared to both patients with chronic pelvic pain and normal controls. The probable source of this was the variation in the muscle tone of the urethra. Unfortunately the relationship between this finding and the pathogenesis of VVS is unknown.

### **Molecular factors**

When the hypothesis of this thesis was developed, little work had been published investigating VVS at a molecular level. However, during the last few years, researchers have investigated possibilities to understand the mechanisms behind vestibular pain.

In 1996, Masterson et al assessed Natural Killer (NK) cell activity in patients with VVS, as a first step in assessing the immune system in these women. They found that women with VVS had decreased NK cell activity compared to healthy controls, although statistical significance was not achieved due to small numbers. They suggested that as NK cells are important in several immunomodulatory mechanisms, including tumour surveillance, control of viral infection and secretion of cytokines, and that some forms of VVS may be associated with Human Papilloma Virus (HPV) infection, that this was the link that may explain the decrease in NK cell activity. Unfortunately the HPV status of their patients was not evaluated, and indeed the role of HPV infection in VVS as a main aetiological factor remains unlikely.

Abnormal neurology of the vestibule has been considered to explain the hyperalgesia. A theory of vulvodynia as primarily a pain disorder secondary to the establishment of a sympathetically maintained pain loop has been suggested. (Cox 1995) This may occur with chronic irritation of any area rich in sensory nerves.

Inflammatory mediators, released by injury or inflammation, activate the unmyelinated C fibre nociceptors and prolonged firing of these nerves sensitise wide dynamic neurones in the dorsal horn. These neurones then respond abnormally to input from mechanoreceptors, and so touch is perceived as pain (allodynia).

Neuroendocrine cell-axonal complexes have been identified in the minor vestibular glands (Warner et al 1996). The authors also demonstrated substance P, a known vasodilator and mediator of neurogenic inflammation, in the axons. They proposed that these findings may explain the exquisite tenderness found in VVS. Increased

number of substance P fibres had previously been found in the bladder mucosa of women with interstitial cystitis (Pang et al 1995).

A later study evaluated nerve fibre density in women with VVS (Westrom et al 1998). Forty-seven patients were assessed and compared to 6 controls, 4 being post mortem samples. They found increased nerve fibre proliferation in the samples from women with VVS and suggested this would provide a morphological basis for enhanced neuronal firing, as suggested in the pain loop theory. Again the comparison was made with interstitial cystitis, where similar results were found (Christmas et al 1990). These findings have been confirmed by Bohm-Starke et al (1998), who showed a significant increase in intraepithelial innervation in women with VVS, suggesting focus should be on the change in the peripheral innervation as a possible explanation for the symptoms of VVS. The authors assumed these free nerve endings were nociceptors, transmitting noxious stimuli, resulting in pain. This assumption was later proved correct when the nerve fibres were neurochemically characterised. (Bohm-Starke et al 1999) They proposed that the crucial question seems to be whether the increase in sensory nerve activity is related to inflammation of the mucosa in women with VVS. Immunohistochemical analysis of the neuroendocrine cells of the minor vestibular glands found the degree of inflammation in vestibular tissue in women with and without VVS was not significantly different (Sloane et al 1998). The authors confirmed the presence of neuroendocrine cells in the minor vestibular glands and that the number of cells expressing the inflammatory mediator serotonin and the chemokine receptor CXCR2 was upregulated by inflammation.

Little work has still been done looking at inflammatory markers in VVS. Foster and Hasday (1997) investigated tissue levels of Interleukin 1  $\beta$  (IL 1 $\beta$ ) and Tumour Necrosis Factor  $\alpha$  (TNF $\alpha$ ) and found elevated concentrations in women with VVS, although paradoxically levels were lowest in the vestibule, the area of highest hyperalgesia. Unexpected findings were recently published showing low expression of inflammatory markers cyclooxygenase and inducible nitric oxide synthase in women with VVS and controls (Bohm-Starke et al 2001). These results indicated there was no active inflammation present in VVS.

This subject will be discussed in greater detail in chapter four.

Genetic investigation appears to have been limited to a single publication. Jeremias et al (2000) examined the relationship between VVS and polymorphisms in the gene coding for IL-1 receptor antagonist (IL-1RA), which is a naturally occurring down-regulator of the proinflammatory response. They found a significant increase in the homozygous form of allele 2 of the gene encoding the IL 1 receptor antagonist. Apparently IL-1RA functions to down regulate IL 1 activity, but in allele 2 positive individuals IL-1 and IL-1RA are upregulated. This results in a net increase in IL-1 bioactivity. When this occurs in women with autoimmune diseases, the inflammatory related symptoms are more severe than in women with the same disease but who lack the IL-1RA 2 allele. The authors suggest this evidence indicates there is a genetic component to VVS, although it is uncertain whether the allele is related to increased susceptibility to development of the syndrome or to the severity of the symptoms, or to both.



In conclusion, the aetiology of VVS still remains a mystery. We can be fairly certain that it is not an infective process and that any possible inflammation is caused by an, as yet unidentified factor. These possibilities raise interesting questions and certain areas remain largely unexplored, in particular the role of hormones in the vestibular area, and whether VVS is indeed an inflammatory condition, both of which warrant further investigation.

## **Treatment of vulval vestibulitis syndrome**

### **Medical Treatment**

It is common for women to have tried a wide variety of treatments prior to attendance to a specialist clinic. Simple measures should always be tried at first but unfortunately are not usually successful.

Early in the history of a VVS an uncontrolled study was published to look at the possible effect of five unrelated drugs, Isotretinoin, Dapsone, Aciclovir, Progesterone and Capsaicin (Friedrich 1988).

Isotretinoin was used in 7 patients, none of whom showed any benefit whilst on the treatment but improved 3 months after cessation of the drug. Unfortunately this drug has major side effects and from this it cannot be concluded whether the effect was due to a delayed effect or whether the patients would have gone into spontaneous remission without treatment.

Dapsone was used in 14 patients but is complicated in that its use needs regular monitoring due to the possible side effect of anaemia. Two patients appeared cured following the treatment, but the majority remained unchanged and one patient's symptoms worsened.

Aciclovir was used in 15 patients: 7 reported a reduction in the severity of their pain and 2 were cured. 5 remained unchanged and 1 patient worsened.

There was no correlation between the response, nor degree of response and the presence of herpes simplex virus antibodies or level of titre.

Progesterone was unhelpful and the majority of the patients experienced vulval burning secondary to application of the cream.

Capsaicin produced severe burning with each application, requiring the use of oral narcotics and no response was reported in half of the cohort. Two patients were cured and 1 showed moderate improvement. The author suggested that VVS represents a form of localised neuropathy, similar to that seen in varicella infection. This argument was based on the fact that aciclovir and capsaicin are effective in treatment of varicella neuropathy, and appeared to have some beneficial effects in VVS.

VVS as a neuropathy was the rationale behind the use of tricyclic antidepressants.

Using a similar regime to that used in dysaesthetic vulvodynia 60% had a positive response (Pagano 1999).

More recently studies have concentrated on a variety of treatments but unfortunately most of the published work is uncontrolled and there is a lack of randomised trials comparing different treatments.

In one study simple measures produced a poor or absent response in the majority of patients, and in the same study, topical steroids only had a moderate or good response in less than 20% of the cohort (Munday and Byrne 1996).

Lubricants (McKay 1985), topical anaesthetics (Turner 1992) antibiotics and steroids appear ineffective in the literature but there have not been any controlled clinical trials (Bergeron et al 1997).

Sonnex (1996) reported treatment with topical steroids, antifungals and intralesional triamcinolone. In his series there was no response to antifungals and again 20% responded to topical potent steroid or intralesional injection of triamcinolone. Three groups were identified depending on the severity of erythema, and unfortunately were not treated with a standard regime. The group who had more severe erythema were pretreated with anti fungals. The groups were too small to show any significant difference between the treatments.

One clinical trial looked at treatment response to topical ketoconazole (Morrison et al 1996). Thirty-four patients were treated with 2% ketoconazole cream three times a day and 44% of their cohort showed an improvement over 8 months. The authors were unsure as to the mechanism by which ketoconazole cream had produced the response, and whether the patients were tested for fungal infection is not mentioned. Although the action is assumed to be anti fungal, ketoconazole does in fact have an anti inflammatory action equivalent to 1% hydrocortisone. It blocks lipoxxygenase and inhibits production of leukotrienes and thromboxanes. They also reiterated the power of the placebo effect and recommended that a double blind controlled trial was needed.

The use of interferon is first line medical treatment in some centres.

Interferon is a cytokine that prevents viral replication by inducing production of intracellular antiviral enzymes and enhancing the activity of natural killer cells against

virus infected cells. Alpha, beta and gamma interferon is available, but beta interferon reportedly has the lowest rate of systemic side effects.

The possible association between VVS and HPV infection encouraged the local application of interferon. The injections were given into the vestibule 3 times per week on 12 occasions, but caused intense pain when introduced and so was discontinued in a large majority of patients. In the ones that persisted with the treatment 15 out of 16 reported relief. All these patients were HPV positive. Interferon was ineffective in patients where HPV was not identified (Horowitz 1989).

A case report was published using interferon by an intramuscular route. The patient was cured and there were reduced side effects compared with local administration (Bornstein et al 1991). This was followed up by a series of 7 women of which 4 gained complete remission, 2 did not respond and 1 had recurrence of symptoms after treatment (Bornstein et al 1993).

A larger series of 55 women was published using intralesional alpha interferon in both HPV positive and negative patients. Just under half reported partial or substantial improvement, but these were all HPV positive women. The author concluded, using a cost analysis, that intralesional alpha interferon treatment as first choice in management of idiopathic VVS, is a cost effective strategy (Marinoff et al 1993).

These accounts do not allow for the varying reports in the literature of the prevalence of HPV infection. Given that many studies have not found an association between HPV and VVS it is difficult to suggest that interferon will be beneficial to many.

One study has been published using methylprednisolone and lidocaine injections to treat VVS (Murina et al 2001). Decreasing doses of both drugs were injected into the vestibule at weekly intervals, for three weeks. A 68% response rate was reported. The method of administration was similar to that used in intralesional interferon injections. The authors suggested this was an effective treatment as methylprednisolone has immunomodulatory and anti-inflammatory properties with lasting efficacy against the chronic inflammation of VVS. They also proposed that lidocaine not only acts as an anaesthetic but also exerts direct action on the vestibular nerve fibres involved in the pathogenesis of the syndrome. Therefore, unfortunately it is difficult to know which treatment is the effective component.

Other less invasive approaches have been suggested, including acupuncture (Secor and Ferrita 1992, Powell and Wojnarowska 1999).

Two studies have been published suggesting that electromyographic biofeedback of pelvic floor musculature is beneficial (Glazer et al 1995, McKay et al 2001).

The first study reported an increase of pelvic floor muscle contraction of 95% with a decrease in resting tension. Instability of muscles at rest was also reduced and subjects reported a decrease in pain of 83% sufficient that 22 out of 28 could resume sexual intercourse. They believed that cutaneous vulval disturbances destabilised pelvic floor musculature and suggested that whatever the initial insult or aetiological factor, vulval vestibulitis syndrome may be a result of autonomically mediated pain.

They concluded that this mechanism as a final common pathway for multiple pathologies and multiple aetiologies, and may explain the lack of consensus on a single antecedant despite consistency in symptomatology of the syndrome.

The second study reported a similar improvement rate of 85% and proposed that treatment to restabilise the pelvic floor muscles has significantly less morbidity than other treatments and the approach is cost effective.

### **Surgical Treatment**

Despite attempts to find a non-invasive, effective solution, surgery still remains the treatment of choice in selected patients with VVS, although success rates vary in published results.

Perineoplasty was initially described as treatment for dyspareunia caused by vaginal outlet distortion (Woodruff et al 1981).

The same author then applied this principle to vulval vestibulitis syndrome patients (Woodruff and Parmley 1983). They described a perineoplasty to include the hymenal ring and 0.5cm of tissue adjacent to this. The incision extends from below the urethra to the fourchette internally, and the lateral incision extends down and terminates above the anal orifice. This includes the fourchette and sometimes the labia minora. The vagina is undermined and exteriorised to be sutured to the skin.

In this series all patients experienced major symptom relief.

Using the same surgical technique, the Woodruff perineoplasty, the study was repeated 5 years later and 13 of 22 patients were much improved. The remainder experienced no relief (Reid et al 1988).

In his initial description of vulval vestibulitis syndrome Friedrich (1987) used excision of the vestibule and vaginal advancement. He excised the hymen and all sensitive areas of the vestibule. Areas anterior to the urethral meatus were excised and left to heal by secondary intention. 56% of the cohort was cured, or reported to be much better.

Marinoff and Turner (1991) suggested that the entire vestibule needed to be removed. They had stricter criteria for entry to surgical procedure and performed a modified Woodruff's perineoplasty, where the outer incision for the paraurethral glands was along Hart's line including the fourchette. Inner incision was behind the hymenal ring. They excised a horseshoe of tissue and mobilised the vaginal mucosa and advanced it to cover the defect. Reported complications were wound haematoma, partial or complete dehiscence of the wound, uneven healing and Bartholin's duct stenosis and subsequent cyst formation.

Vestibuloplasty, during which the tissue is undermined but not excised, was suggested as an alternative approach. However in a study comparing the two procedures no relief was found with vestibuloplasty, whereas 9 out of 11 patients were cured with perineoplasty (Bornstein et al 1995). He speculated the reason of failure of vestibuloplasty as reinnervation following denervation and subsequent hypersensitivity. It was suggested that given denervation did not improve symptoms



initially that innervation disturbances are not a direct cause of VVS. He concluded that vestibuloplasty is not an acceptable procedure for treatment of VVS.

Attempts have been made to simplify the surgery.

One study suggested that limits of excision should be the areas where tenderness was elicited. Success rates of 75% were reported and procedures varied from posterior hymenectomy to posterior vestibulectomy and removal of the paraurethral areas (Goetsch 1996).

A modified vestibulectomy was presented where the superior excision margins were limited to halfway up the inner aspect of the labia minora with or without a modified Fentons procedure. 59% had a complete response, 30% had a partial response and 11% had no response (Kehoe and Luesley 1996). Although proposed as an alternative, the success rate is lower than that of full vestibulectomy and no information is given about long term follow up and recurrence rates.

The use of the LASER has been suggested as treatment for vulval vestibulitis syndrome: Laser vulvectomy for vulval vestibulitis syndrome was reported to have inconsistent results but improvement was shown in up to 60% (Davis 1989).

A small series was presented where laser ablation of the vestibule was performed.

These women were slow to heal, many taking many months (Kaufman and Friedrich 1985). The most detailed account of laser surgery used laser ablation of the inflamed areas. In patients with tenderness over the duct areas only, laser produced a good

result. If tenderness extended further than the ducts laser did not produce a significant improvement (Michelwitz et al 1989). Patients who underwent perineoplasty had an elliptical specimen excised, encompassing the area between the hymenal ring and the perineal body ridge and extending to both Bartholins ducts. All of these patients improved.

Treatment protocols remain substandard, with surgery still being the only option in women who do not respond to medical treatment.

A recent review of non surgical treatment suggested tricyclic antidepressants as first line treatment with biofeedback and psychological counselling, with non responders proceeding to surgery. This review reiterated the need for randomised multicentric clinical trials (Mariani 2002).

The lack of randomised trials was commented upon by Bergeron et al (2001) when they performed a randomised trial of vestibulectomy, electromyomgraphic biofeedback and group cognitive-behavioural therapy.

In this study 78 women of the original 87 completed the study as 7 assigned to vestibulectomy dropped out before treatment, as did 1 each assigned to biofeedback and behavioural therapy. The authors argue that this may influence their findings: that vestibulectomy was significantly more successful than other groups, although all three groups improved with treatment. However, they also performed a posteriori intent-to-treat analysis to account for the pre-treatment drop outs, but found that the general pattern of results was confirmed.

They concluded that “multimodal treatment approaches may be essential to achieve significant improvement in all aspects of the disorder and that cognitive behavioural therapy and biofeedback represent promising alternatives to vestibulectomy”.

## **Objectives of thesis**

VVS continues to pose challenges. At present our diagnosis, assessment and evaluation of response to treatment is subjective. Our choice of treatment is universally random, as we are unsure about the underlying pathological process we are treating.

From the literature, basic questions have been raised as to the nature of this syndrome. It has always been assumed that VVS is a chronic inflammatory condition but anti-inflammatory treatment has been unsuccessful. Other perspectives, such as possible roles of hormonal components have not yet been investigated.

Until our understanding of the aetiology of the complex condition improves we are unlikely to be able to improve our standard of care for these women.

In view of these problems, the objectives of this thesis are:

1. To define the epidemiological characteristics of a selected cohort of patients and compare these to existing data. i.e Are there patterns or characteristics that further define VVS patients and their responses?
2. To objectively quantify the pain experienced in VVS. i.e. Is the use of an "algometer" valuable in assessing and following patients with VVS?
3. To explore whether VVS is an inflammatory condition. i.e. What is the expression of inflammatory cytokines in tissue of patients with VVS?
4. To explore whether VVS has a hormonal component. i.e. What are the oestrogen levels of patients with VVS, and are there altered expression of oestrogen receptors in tissue of these patients?

## **CHAPTER TWO - ASSESSMENT OF VULVAL VESTIBULITIS SYNDROME**

Diagnosis and assessment of vulval disease is often considered to be a difficult area of general gynaecology. Frequently the diagnosis is apparent if a detailed history has been taken, but often women do not volunteer relevant information, particularly that of a sexual nature, and may need to be prompted by the clinician. Women may not have presented for many years due to fear or embarrassment, and it is important to build a good rapport quickly.

In a Vulval Clinic, women may arrive with cynical views as many have had misdiagnosed symptoms for years and will have seen multiple clinicians.

A sympathetic and comfortable environment for taking a history and examination is essential.

Assessment of VVS is particularly difficult as physical signs are often minimal, or the condition is sufficiently severe that patients are unable to tolerate examination.

Patients with VVS will often give the diagnosis in their history. The classic story is of a young woman in her twenties or thirties who has had normal sexual relationships in the past. She then describes sudden onset of superficial dyspareunia, often without identifying a precipitating factor, but often blaming candidal infection. The pain is sharp and precipitated by attempted penetration at intercourse, but then often leads on to a chronic vulval burning that may last hours or days. Frequently the pain is so severe as to prevent intercourse completely. When questioned these women also report difficulty in tampon use and have changed to sole use of sanitary towels.

With the diagnosis of VVS in mind it is important to examine the patient, to exclude other causes of vulval pain and to confirm the physical findings of VVS.

### **Examination of the vulva**

The optimal situation in which to confirm the diagnosis of VVS is a specialised Vulval Clinic, ideally staffed with a dedicated nurse, which provides a private examination room, and not just a curtain pulled round in the corner of the gynaecology clinic.

In our Vulval Clinic we have an examination couch that tilts back to 90 degrees placing the patient into the lithotomy position. It is essential to have good lighting and the vulva should be examined both with the naked eye under a bright directional lamp and with use of the colposcope.

Use of the colposcope in vulval disease is becoming more common and enables a magnified view of the vulva which makes subtle changes more obvious and allows more accurately directed biopsy if needed.

Our colposcope is connected to closed circuit television, which allows the patient to see the area being examined and enables her to point out any areas that are symptomatic.

Although, as previously described in the introduction to this thesis, acetic acid and toluidine blue stains are useful in identification of abnormal areas in other forms of vulval disease; they do not have a role in the diagnosis and assessment of VVS.

In VVS there may be minimal physical signs, the changes that do appear are confined to the vestibule with no abnormality elsewhere on the vulval skin. This explains why VVS is often misdiagnosed, as even in a gynaecology clinic it is difficult to adequately view this area and the abnormalities seen are often subtle.

The usual findings in a patient with VVS are erythema of varying degrees within the vestibule and apparent enlargement of the openings of the greater and lesser vestibular glands.

Occasionally it is necessary to biopsy the vestibule, to exclude other vulval pathology or for research purposes. This is performed in the Vulval Clinic using the same technique for other areas of the vulva (MacLean and Reid 1995).

Local anaesthetic, usually 3% Citanest (prilocaine 30mg/ml) plus Octapressin (felypressin 0.03iu/ml) contained within a 2.2ml cartridge is injected, by use of a dental syringe using a 27-gauge needle into the area to be biopsied.

This is a quick acting agent and the biopsy is then taken using a 4mm Stiefl punch biopsy. The core of tissue is then removed and the haemostasis achieved at the base of the wound by application of silver nitrate sticks or Monsel's solution (Ferric subsulphate).

VVS is a chronic condition, usually with symptoms persisting for more than six months, with many patients reporting symptoms lasting many years. However, an allergic reaction, or an infective agent can cause an acute vestibulitis. Therefore in the assessment of a patient with possible VVS these factors need to be excluded.

In our clinic all patients with a history indicative of VVS will have a complete set of swabs taken at initial assessment: vaginal and endocervical swabs are collected for trichomonas, gonorrhoea, yeast and chlamydia infection. These are sent for laboratory analysis. Bacterial vaginosis can be tested for in the clinic by application of 10% potassium hydroxide solution to a vaginal swab and smelling for the presence of amines. Unfortunately patients with severe vestibulitis may not be able to tolerate a speculum examination, in which case low vaginal swabs are taken, as well as urethral swabs for chlamydia.

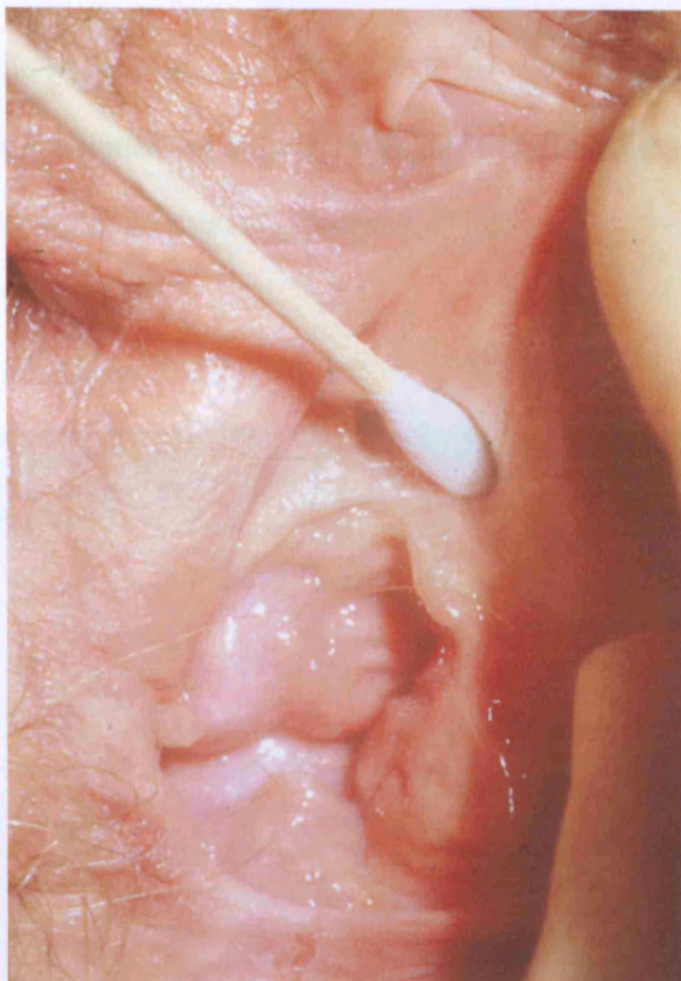
### **Assessment of vestibular tenderness**

One sign that is always easily elicited in patients with VVS is tenderness over the entrance to the vestibular glands. Traditionally the method of eliciting and assessing tenderness was by use of a cotton tip. Goetsch (1991) suggested rolling a Q tip over the entrance to the vestibular glands to provoke the pain described with attempted penetration. This is shown in Fig 2.1.

There are many problems associated with this method. It gives the clinician the answer to the question of whether or not tenderness is present, but is subjective and does not quantify the tenderness elicited. Equally, it is not a reproducible method of assessment as it is completely operator dependent. Different clinicians will apply different pressure and even the same clinician is unlikely to be able to reproduce the same level of force over different vestibular areas, let alone at subsequent clinic visits.



Fig 2.1 Q tip application



This method is not recordable, except as pain being present or absent, and therefore subtle response to treatment is difficult to ascertain as the clinician is relying on the patient's memory of previous visits.

## **Algesiometry**

To overcome these problems the vulval algesiometer was developed by the Departments of Genito-Urinary Medicine and Biomechanical Engineering in Freedom Fields Hospital in Plymouth.

The algesiometer consists of a hand held probe that includes a metal thrust probe contained within a plastic-casing sleeve. (Fig 2.2)

Fig 2.2 The algesiometer



The sleeve serves a dual purpose: flattening the skin surrounding the vestibular gland and therefore enabling accurate aim over the entrance to the gland itself, and secondly when pushed back it activates the sensor which causes the thrust probe to fire. The hand held probe is connected to a main control unit that is powered by mains

electricity. The control unit allows the force of impact and the frequency of firing of the thrust probe to be altered.

The force readings on the algometer correspond to pre-set levels of force measured in milli Newtons and the machine has been calibrated accordingly. The control unit is connected to a foot pedal that allows the force to be increased whilst examining the patient.

The LCD circuit used gives a reading of 0 a finite value in milli Newtons. Therefore for statistical analysis all readings are increased by 1. The force value for each reading is shown in the table 2.1. If the patient does not feel discomfort with a force reading of 7 then she is said to have scored a maximum of 8 for that area.

Table 2.1 Force settings for the algometer

<b>Force setting</b>	<b>Measured force (mN)</b>
0	178
1	356
2	578
3	756
4	1068
5	1334
6	1646
7	2046

The initial work using the algometer, published in 1996 showed that patients with VVS achieved lower readings than a normal control population (Curnow et al 1996).

The lower readings corresponded to decreased force exerted by the algometer. Therefore the more tender the vestibular area the less force could be tolerated. Further work showed assessment of the vestibule within the condition when treated with ketoconazole. That study looked at the improvement of the algometer readings during treatment with ketoconazole, but did not comment on the patients' symptomatology (Morrison et al 1996).

In view of these factors we decided to look at the algometer readings of women before and after treatment, to see whether it correlated with the patients' subjective view of symptom severity.

## **Study**

In the group of women who had improved with treatment, we wanted to see whether the algometer readings would correlate with the improvement in patients' symptoms.

Improvement was defined as the point at which the patient was no longer symptomatic and able to tolerate penetrative intercourse.

Before participating in this study all patients had a full set of swabs taken to exclude chlamydia, gonorrhoea, candida, trichomonas and bacterial vaginosis.

All patients had been examined to exclude other vulval pathologies.

## **Method**

Fifty-five patients attending the Vulval Clinic with a diagnosis of VVS of more than six months duration were assessed using the algometer.

Of this group 9 patients only had an initial assessment reading taken. Thirty patients had sequential readings before and after treatment and of these 25 reached the point of discharge from the study. Eleven patients only had a single reading after treatment and 5 had sequential readings after different treatments but no initial assessment.

This was due to different referral patterns and was dependent on treatment used prior to the Vulval Clinic visit.

The treatment protocol followed in this clinic was 2 months topical application of Trimovate® cream, followed by 2 months topical application of Premarin® cream, if there had been no response to the initial treatment.

For the purpose of this study the thrust probe of the algometer was set at a frequency of 0.5 seconds, this meant that a single thrust of the probe was delivered over the selected vestibular area. This was thought to be more representative of the pressure applied during intercourse, rather than the continual tapping action that would be reproduced using a higher frequency of thrust.

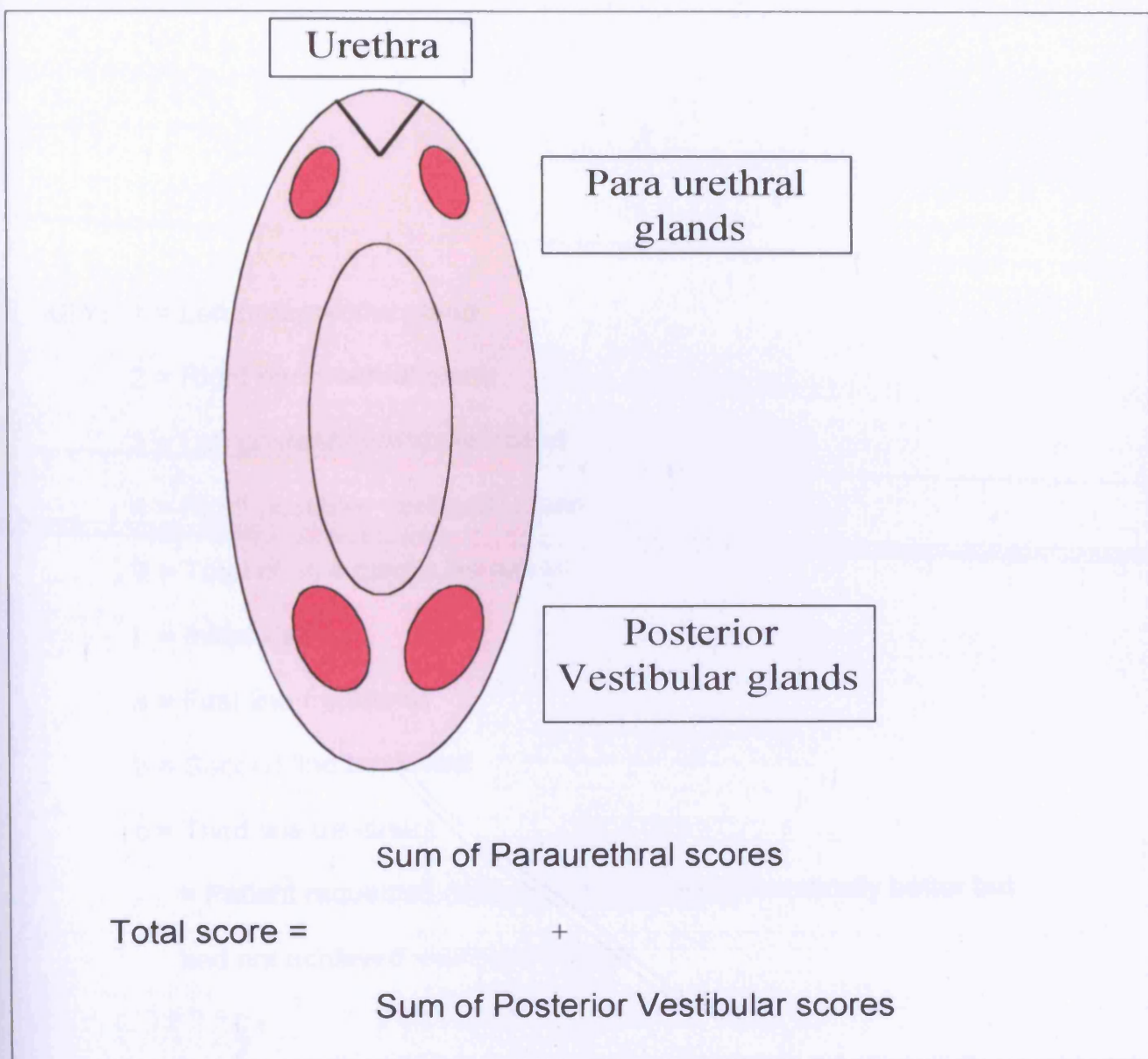
Individual readings were recorded from the paraurethral glands and the two posterior vestibular glands (Morrison et al 1996). The sum of these readings gave a final score for that visit.



Each of the four vestibular areas were assessed in order, starting with the probe firing with minimum force and increasing the force by one reading at each application of the probe. The force was increased until the patient reported that the pain provoked was representative of that pain produced by coitus.

The readings for each area and the total for that visit were recorded on a vulval map, as shown in Fig 2.3.

Fig 2.3 Positions of algesiometer readings



## **Results**

Following treatment, the patients returning to clinic were reassessed using the algometer in the same way.

Table 2.2 shows the individual readings of the algometer probe for each of the four glands tested at each visit after different treatments.

KEY: 1 = Left paraurethral gland

2 = Right paraurethral gland

3 = Left posterior vestibular gland

4 = Right posterior vestibular gland

T = Total of all 4 glands assessed

I = Initial visit

a = First line treatment

b = Second line treatment

c = Third line treatment

- = Patient requested discharge as was symptomatically better but  
had not achieved maximum scores

Table 2.2 Algesiometer readings

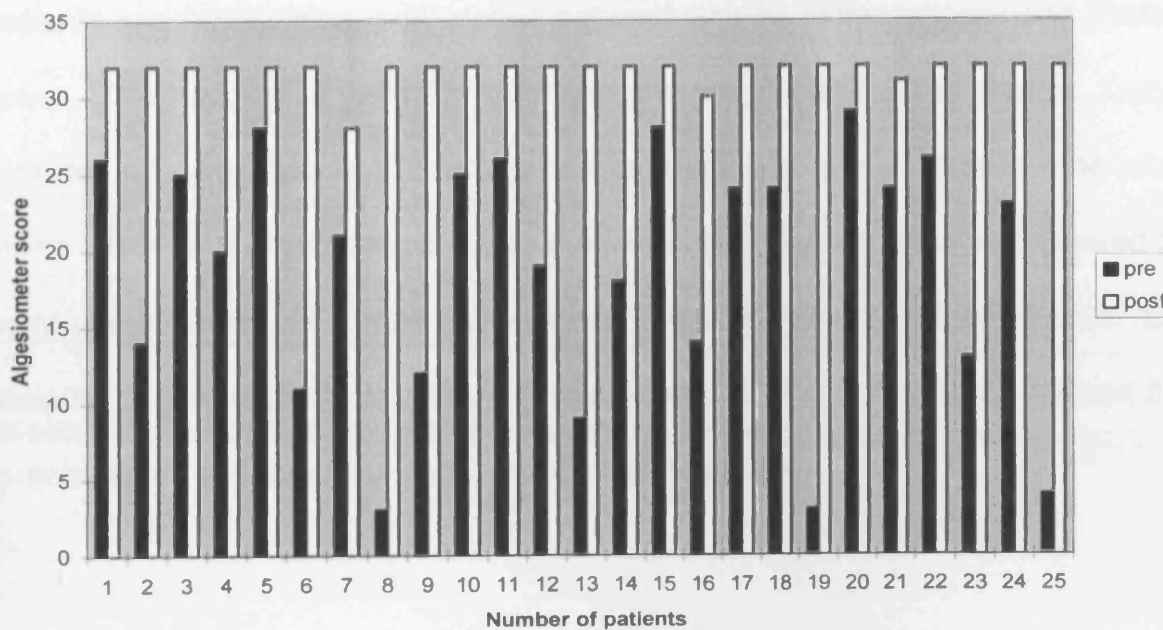
Pt	1l	2l	3l	4l	5l	1a	2a	3a	4a	5a	1b	2b	3b	4b	5b	1c	2c	3c	4c	5c
DA						8	8	8	8	32										
CB	8	8	7	5	28															
LB						7	7	7	5	26	8	8	8	8	32					
HB	4	4	3	3	14	8	8	6	6	28	8	8	8	8	32					
MC	1	0	1	3	5	2	2	3	3	10										
MC						0	0	0	0	0										
DC						7	7	4	7	25	8	8	8	8	32					
KD						5	5	5	5	20	8	8	8	8	32					
JD	0	1	1	1	3	8	8	8	7	31	-	-	-	-	-					
MD						8	8	8	8	32										
MF	3	3	8	8	22	3	3	8	8	22										
LF	2	3	4	2	11											8	8	8	8	32
VG	7	7	7	7	28	8	8	7	7	30										
DG	3	3	2	3	11	7	7	5	5	24	7	7	5	4	23					
SG						8	8	8	8	32										
JH	8	8	7	7	30															
EH	8	6	4	4	22	6	6	6	5	23										
GH						3	7	2	1	13						8	8	8	8	32
VK						8	8	8	8	32										
EL	7	7	4	3	21	7	7	7	7	28	-	-	-	-	-					
ELJ	0	1	1	1	3	8	8	8	8	32										
JL	3	3	3	3	12															
KL	2	3	6	6	17	3	3	3	3	12	8	8	8	8	32					
KL	3	4	1	2	10	4	4	3	3	14	4	4	3	3	14					
AL	0	0	1	1	2															
JL	6	7	5	7	25	8	8	8	8	32										
VM						7	7	7	7	28										
HM						8	8	3	7	26	8	8	8	8	32					
AMG						8	7	7	7	29										
CM	7	7	8	8	30															
TM	7	7	1	2	17	8	8	8	8	32										
CND						4	7	3	1	15										
JN	4	7	5	6	22	8	8	7	8	31	8	8	8	8	32					
JP	7	7	7	7	28	8	8	8	8	32										
LP	7	7	5	5	24	7	7	7	7	28	8	8	8	8	32					
SP	0	0	0	0	0	5	3	2	2	12										
SR						8	8	8	8	32										
SR	1	1	1	6	9	8	8	8	8	32										
SR	3	2	3	2	10															
LS	5	6	6	6	23	3	5	5	6	19	8	8	8	8	32					
SS	7	7	7	7	28	8	8	8	8	32										
LS	7	7	7	2	23															
HS	7	7	2	7	23															32
JS																				32
AT	0	0	0	0	0															
AT	8	8	1	5	22	8	8	7	8	31										
LT	7	5	7	5	24															
AT	1	1	1	1	4						8	8	8	8	32					
SV	7	7	5	7	26	8	8	8	8	32										
JV	7	7	7	3	24	8	8	8	8	32										
SW	0	1	1	1	3	3	5	8	8	24										
CW	3	3	3	1	10	8	8	7	7	30										
TW																				32
LW	1	7	2	4	14						8	8	8	8	32					
GW	7	4	6	7	24															32



In order to evaluate the correlation between subjective assessment by the patient and objective assessment by the algometer, we looked at the patients whose symptoms had improved sufficiently to be discharged from the clinic and analysed their algometer scores before and after treatment. This comprised a group of 25 patients.

These results are shown in Fig 2.4.

Fig 2.4 Algometer readings before and after treatment



The results show that there is correlation between algometer readings and the patients' subjective reports on the progress of their symptoms. Patients whose symptoms were initially bad had low algometer readings. Those who reported an improvement in their symptoms also had an improvement, i.e. rise in their algometer scores.

There were patients who did not respond to treatment. Their mean pre treatment scores were 11 and mean post treatment scores were 18. These results have not been included in the chart. Response to treatment is discussed in a later chapter.

Individual scores of each of the areas were recorded to see if there was a difference between anterior paraurethral and posterior vestibular areas.

In the total group tested at their initial visit, symptoms were worse in the anterior glands in 4 patients and worse in the posterior glands in 11 patients. Twenty-four patients had no significant difference between scores of the anterior and posterior areas. The majority of patients were symptomatic in both sets of glands. Only 3 reported no symptoms in the anterior areas and 2 had no symptoms in the posterior areas. This lack of pattern was also evident in the group of 25 who improved to the point of discharge. Of the 25 patients only 3 had worse anterior symptoms. Eleven patients had no difference between their anterior and posterior readings and the remaining 11 patients had worse symptoms posteriorly.

### **Statistics**

The differences in the scores before and after treatment were analysed using the Minitab Statistics package for Windows. Non parametric data were analysed using the Mann Whitney U test. Results are shown in Table 2.3

Table 2.3 Statistics for algometer readings before and after treatment

	Pre treatment	Post treatment
Median value	21	32

95 % Confidence Intervals	- 17.98 to -7.0
p value	0.0001

A significant difference was found between the algometer readings before and after treatment. As this was related to the improvement in the patients' symptoms, this shows that there is good correlation between the algometer readings and patients symptomatology.

## **Discussion**

These findings are important when planning surgical management of cases that are resistant to medical therapy, as it would appear that in the majority of women with VVS there is involvement of all of the glands within the vestibule.

This appears to contradict a recent report arguing for a more conservative surgical approach and not including the paraurethral areas in excision margins (Kehoe and Luesley 1998).

We found that the vulval algometer gave us the first reported method of quantifying tenderness in VVS.

It gives us a definitive measurement of tenderness for each clinic visit. These readings are recordable and therefore comparable at subsequent visits. It also allows different colleagues who may see the patient, a method by which to assess improvement or deterioration, and therefore removes the need for the patient to see the same clinician at subsequent appointments.

This method was well tolerated and accepted by patients. They found it advantageous to see improvement in their readings and with treatment and thus the technique provided a form of biofeedback. Often the women seen in our clinic have had symptoms for many years and they may not remember what is 'normal'. Many of our patients have developed a cycle of avoiding intercourse as they remember it to be so painful following development of their vulval symptoms, and so are reluctant to try and resume a normal sexual relationship until they have been encouraged to do so by the doctor. The algometer provides them with the necessary encouragement, as they visualise that they can now tolerate a much greater force than was possible prior to treatment.

## **Conclusion**

For the clinician the algometer gives a method of quantifying the pain and comparing response to treatment and so allows comparison of different types of treatment. The vulval algometer is used as a non-invasive method of assessing and quantifying vestibular tenderness. It is simple to use, gives a recordable result and provides the clinician with a reliable method of assessing treatment progress.

The vulval algometer has been used in several aspects of the work contained in this thesis and will be referred to in later chapters when assessing patient response to treatment within our cohort of women with VVS.

### **CHAPTER THREE - EPIDEMIOLOGICAL CHARACTERISTICS OF STUDY PATIENTS WITH VULVAL VESTIBULITIS SYNDROME**

VVS continues to be a condition of unknown aetiology. In an attempt to identify causal relationships, efforts have been made to identify the population of women that this condition affects.

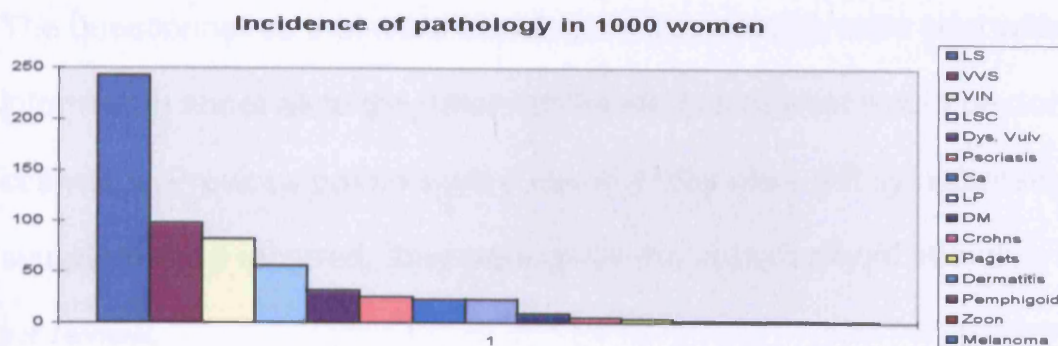
Epidemiological studies have been performed in the USA (Goetsch 1991, Peckham et al 1986) and in Sweden (Sjoberg and Nylander 1997), which have compared a cohort of patients with VVS to a normal population, to try and define characteristics unique to this group of women.

The epidemiological characteristics of British patients with VVS has never been investigated and we recognise different cultural factors may apply.

In the population of women attending the Vulval Clinic at the Royal Free it appeared that many of the characteristics defined in the literature were exhibited by our patients. Therefore we surveyed our population who had been diagnosed with VVS and compared our profile with other published data of epidemiological surveys to see if our cohort was typical.

Patients with vulval pain represent approximately 13% of attenders at the Royal Free Vulval Clinic (MacLean et al 1998) which sees approximately 300 new referrals per year (Fig 3.1). Of these just under 10% have VVS, with the remainder being diagnosed as dysaesthetic vulvodynia. In subsequent years the percentage of women presenting with VVS has increased slightly, to around 15%

Fig 3.1 Incidence of vulval pathology in 1000 women



## Method

Between August 1996 and August 1998 all new patients who fulfilled Friedrich's criteria for VVS were invited to complete a questionnaire. In addition to this all patients who had been diagnosed with VVS in the previous 3 years were sent the questionnaire by post. In total, 110 questionnaires were sent.

The questionnaire (Appendix 3.1) was divided into 8 sections: general demographic information, symptomatology, menstrual history, contraceptive history, sexual history, obstetric and gynaecological history, past medical history and a general section covering allergies, family history and diet.

The telephone number of the investigator was included so if there were any queries the patient could contact us.

Patients who were given the questionnaire in the clinic were given the opportunity to complete it during their visit, or could choose to complete it at a later date and return it by post.

The questionnaires that were sent to previous patients were sent with a covering information sheet as to the nature of the study and what would be done with the data collected. Previous patients were asked if they were still symptomatic. If so, or if their symptoms had recurred, they were given the opportunity of attending the Vulval Clinic for review.

Some patients who were successfully treated declined to participate in ongoing research, as they thought we would only be interested in patients who still had symptoms. A return envelope was included to facilitate replies. Approximately 10% of the questionnaires were returned by the Post Office to sender. This is not unusual as our cohort is typical of the mobile population within large cities.

Sixty-two questionnaires were returned by the patient, but one had not been fully completed and so was discarded. This gave a completion rate of 56%. The remaining 61 were entered into a database using Microsoft Access and the results analysed.



## **Results**

### **Demographic details**

All the patients were Caucasian except one of Asian origin. This ethnic distribution is not representative of our general gynaecological referral pattern, or of referrals to the Vulval Clinic.

The median age of our cohort was 34.6 years with a range of 22 to 53 years.

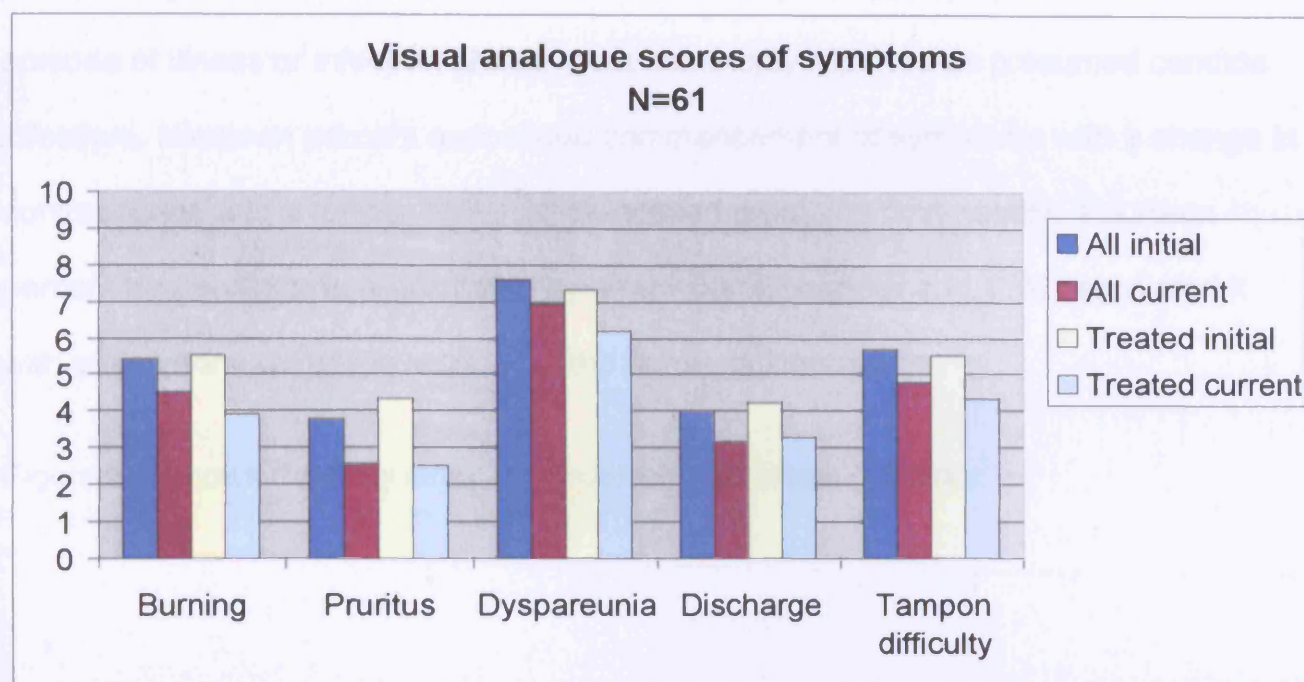
Approximately half of the referrals were from General Practitioners and half were tertiary referrals from consultant gynaecologists, dermatologists and genito-urinary medicine physicians. Approximately 60% of the referrals were from the London area, with the remainder from throughout the UK and 2 patients from abroad.

### **Symptoms**

The mean duration of symptoms was 4.5 years (range 9 months - 20 years). Patients were asked about different symptoms and requested to assess the severity of their symptoms at the onset of the condition and the current severity of symptoms using a visual analogue score, on a scale of 0 - 10. Some of the patients were new referrals and so there was no difference in their analogue scores. Some of the cohort had been treated successfully and so overall their scores had decreased.

The mean visual analogue scores of symptoms, at initial presentation and current symptoms, of the whole cohort, and those who had been treated or were undergoing treatment (excluding the new referrals whose scores were the same), is shown in Fig 3.2.

Fig 3.2 Visual analogue scores of symptoms



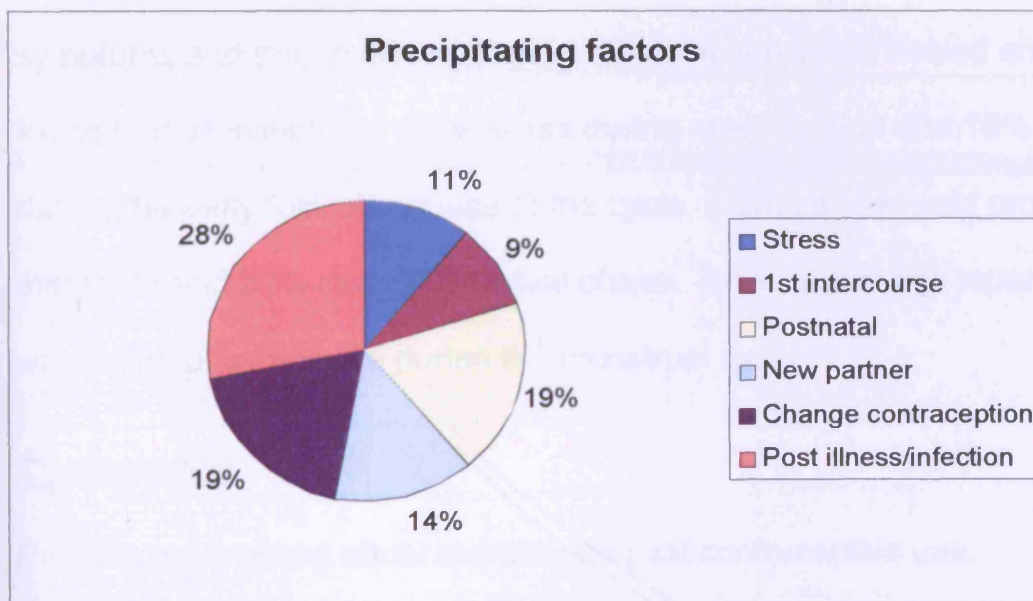
It is not surprising that the most troublesome symptom was entry dyspareunia, followed by difficulty inserting tampons. Some patients had experienced increasing pain or difficulty with coitus or tampon use, to the extent that they were unable to continue. Nevertheless they rated the severity of symptoms as the same from onset of symptoms to presentation at clinic.

Seventy-six percent of the cohort said that since developing VVS their symptoms had been constant rather than intermittent, and symptoms were precipitated with every attempt at intercourse. Many experienced post coital burning that could last several days after coitus.

Eighty-five percent of the cohort could identify a precipitating factor or event prior to which they had been asymptomatic. Five of the patients suggested multiple precipitating factors. The most common event that preceded symptoms was an episode of illness or infection (28%), most commonly reported as presumed candida infection. Nineteen percent associated commencement of symptoms with a change in contraception and a further 19% had developed problems post natally. Fourteen percent had symptoms precipitated by a new sexual partner and 11% associated it with stress, for example at work or in the home, or depression.

Figure 3.3 shows the frequency of perceived precipitating factors.

Fig 3.3 Precipitating factors



## **Hormonal Factors**

### **Menstrual history**

Eighty-six percent of the cohort was premenopausal and 14% post menopausal.

The mean menarche was 13 (range 9.5 - 16). Sixty-eight percent were having regular menses, of which 39% were controlled by the oral contraceptive pill; the mean cycle length was 24.2 days (range 18 - 40). Of the 8 postmenopausal patients, 50% used to have regular menses, with the other half having had irregular cycles (range 18 – 45).

A quarter of the patients suffered from dysmenorrhoea. Less than half of the cohort (45%) regularly used tampons. Discounting the postmenopausal patients, 3 chose not to use tampons and the remainder found their use painful.

These results are summarised in Fig 3.4

The cohort was asked if they noticed any relationship between the severity of symptoms and their menstrual cycle. Forty-five percent denied any relationship, 18% found that all symptoms were worse during menstruation and 18% noticed a change during the early follicular phase of the cycle. Eight percent said problems increased mid cycle and 22% during the luteal phase. Several patients reported bimodal worsening of symptoms during the menstrual cycle.

### **Contraception**

Patients were asked about current and past contraceptive use.

Thirty percent had abandoned using any contraception. Thirty percent were currently using the combined oral contraceptive pill (cOCP) and 70% had used it in the past.



Over half (52%) had previously used condoms but only 17% were currently using them.

A summary of past and present contraceptive use is shown in Fig 3.5.

Fig 3.4 Menstrual history

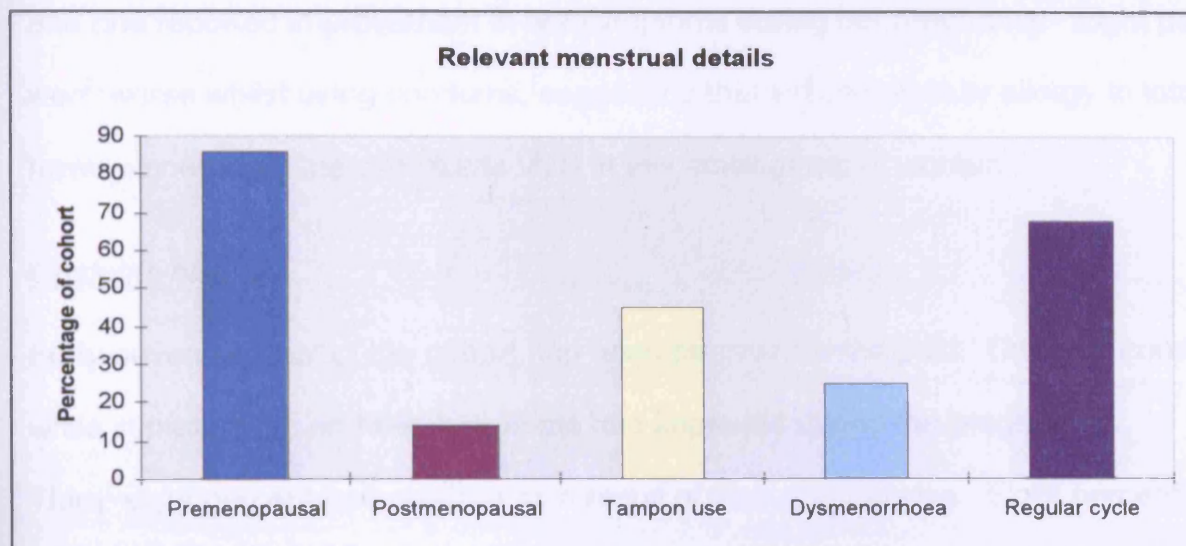
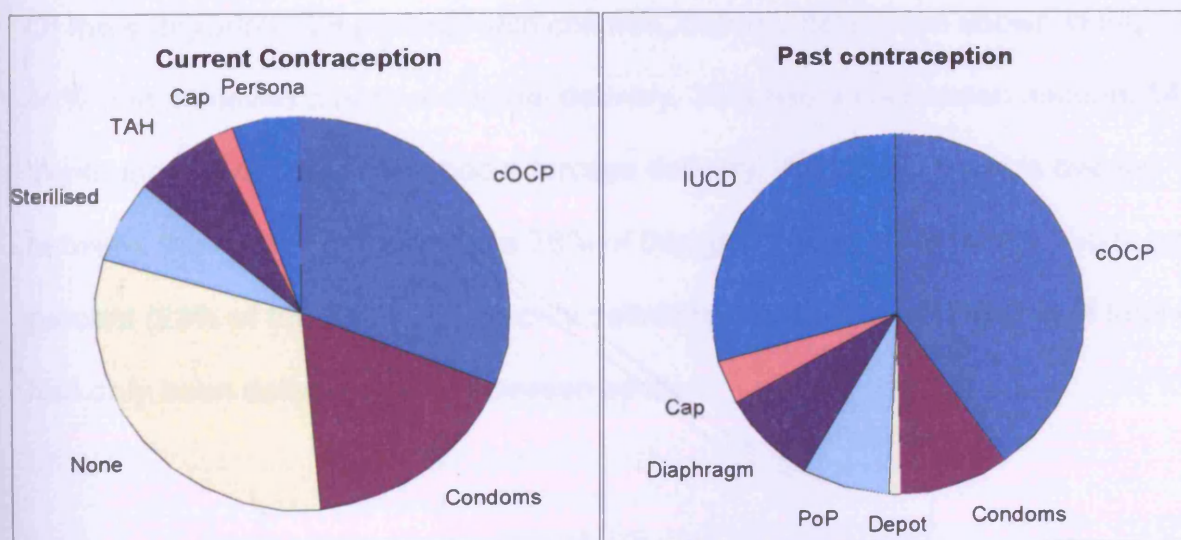


Fig 3.5 Contraceptive use



When asked whether they thought that their contraception bore any relation to their symptoms (19% had associated a change in their contraception with commencement of symptoms), 35% reported change in symptoms related to type of contraception used. The most frequent response (18%) was symptoms worsening whilst taking the combined oral contraceptive pill (cOCP). Two patients had improved on the cOCP and one reported improvement in her symptoms during her pregnancy. Eight percent were worse whilst using condoms, suggesting that inflammation or allergy to latex may have exacerbated the symptoms VVS in this small group of women.

### Obstetric history

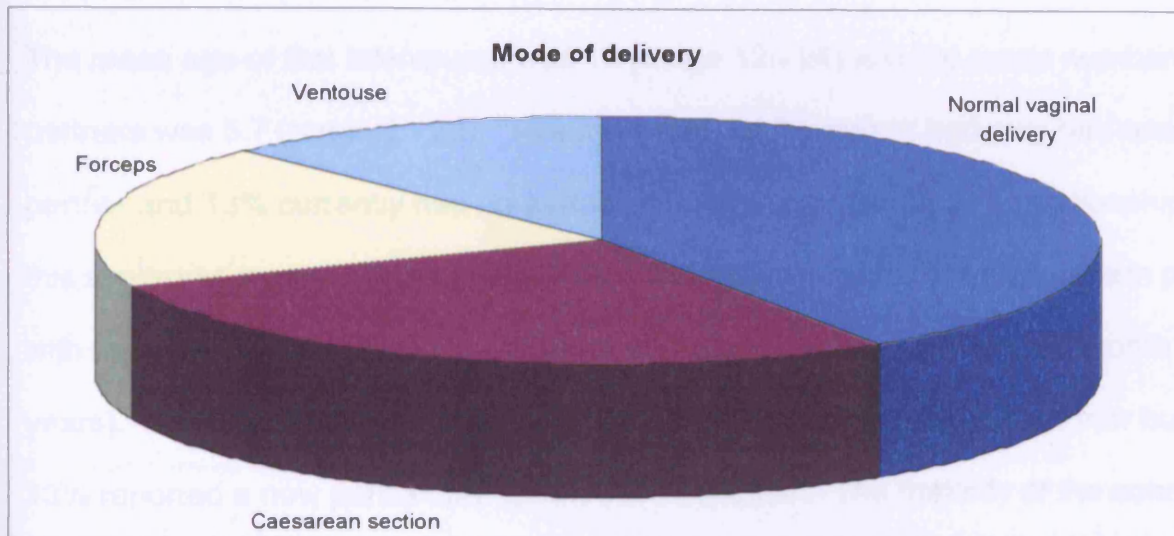
Forty-seven percent of the cohort had been pregnant in the past. One had conceived while symptomatic and her symptoms had improved during the pregnancy.

Thirty-eight percent had children as a result of their pregnancies. Eight percent were primiparous and 30% were multiparous. Seventeen percent had had miscarriages and 12% had had terminations of pregnancy. One patient had had an ectopic pregnancy.

Of the subgroup of 28 patients with children, delivery details are shown in Fig 3.6:

46% had achieved a normal vaginal delivery, 32% had a Caesarean section, 14% had Ventouse delivery and 10% had a forceps delivery. Obviously there is overlap between the modes of delivery as 78% of this group were multiparous. Sixty-one percent (23% of total cohort) had only delivered vaginally and 17% (7% of total cohort) had only been delivered by Caesarean section.

Fig 3.6 Mode of delivery



Three quarters of vaginal deliveries had caused perineal damage. Thirty-two percent of this subgroup had an episiotomy and 46% a tear. Just under a third of the subgroup had wound healing difficulties, but this represents only 15% of the total cohort.

Sixty-four percent of the subgroup breast-fed, with the mean duration of 7.1 months (range 1 - 36 months). Eighty-seven percent resumed intercourse post natally and the mean time to resume intercourse was 6.6 months (range 1 - 60 months). Thirteen percent of those who had children, have not had intercourse since, but none of these had initially developed symptoms post natally.

## **Sexual Factors**

### **Coitus**

The most striking result of this section is that 50% of the cohort was no longer able to have sexual intercourse.

The mean age of first intercourse was 18 (range 12 - 24) and the mean number of partners was 5.7 (range 1 - 22). Twenty percent of the cohort had only had one partner and 13% currently had no partner - the stresses placed on a relationship by this syndrome was mentioned frequently. The majority had a single long-term partner with the mean length of the current relationship being 7.6 years (range 1 month - 29 years). Thirteen percent had experienced symptoms with a previous partner but only 13% reported a new partner precipitated the condition. The majority of the cohort had previously had intercourse with their current partner without difficulty. The mean frequency of intercourse a week was 2.7 times prior to having VVS and had dropped to 0.6 times per week currently, with half of the cohort no longer able to tolerate penetrative intercourse. These results are summarised in Table 3.1

Table 3.1 Sexual history

Mean age of 1st intercourse	18.1 (range 12 - 24)
Mean number of partners	5.7 (range 1 - 22) 20% only had single partner
Mean time with current partner	92.6 months (range 0 - 350) 13% currently have no partner
Symptoms with previous partner	13%
Mean frequency of intercourse	Pre symptoms: 2.7 / week Post symptoms: 0.6 / week



### **Infections**

When questioned about previous infections, 85% said they had candida infection in the past, but the majority of these had not had confirmatory microbiology. Known HPV infection rate was 8% and 7% had previous chlamydia infection. Eighteen percent had been diagnosed with bacterial vaginosis. Two patients had vulval herpes simplex and 2 had Trichomonas. Two patients had previously had vulval warts. There were no cases of gonorrhoea.

### **Previous history**

#### **Gynaecological**

Thirty-eight percent of the cohort had other gynaecological problems in the past. 10% had been diagnosed with polycystic ovaries and 7% with endometriosis. Eight percent had a laparoscopy to exclude other pathology without a diagnosis being made. Three percent had previous pelvic inflammatory disease and 5% had ovarian cysts. One patient had a premature menopause and prolapse and one had a fibroid uterus.

Eighteen percent had previously had an abnormal smear and the mean duration since last smear was 21 months (range 1 - 144 months)

#### **Medical**

Thirty-eight percent of the cohort had experienced other skin problems with eczema elsewhere on the body being the most common (20%). Twelve percent suffered with acne and 3% had psoriasis on sites other than the vulva.

### **Family**

Two patients reported female relatives with vulval problems.

### **Diet**

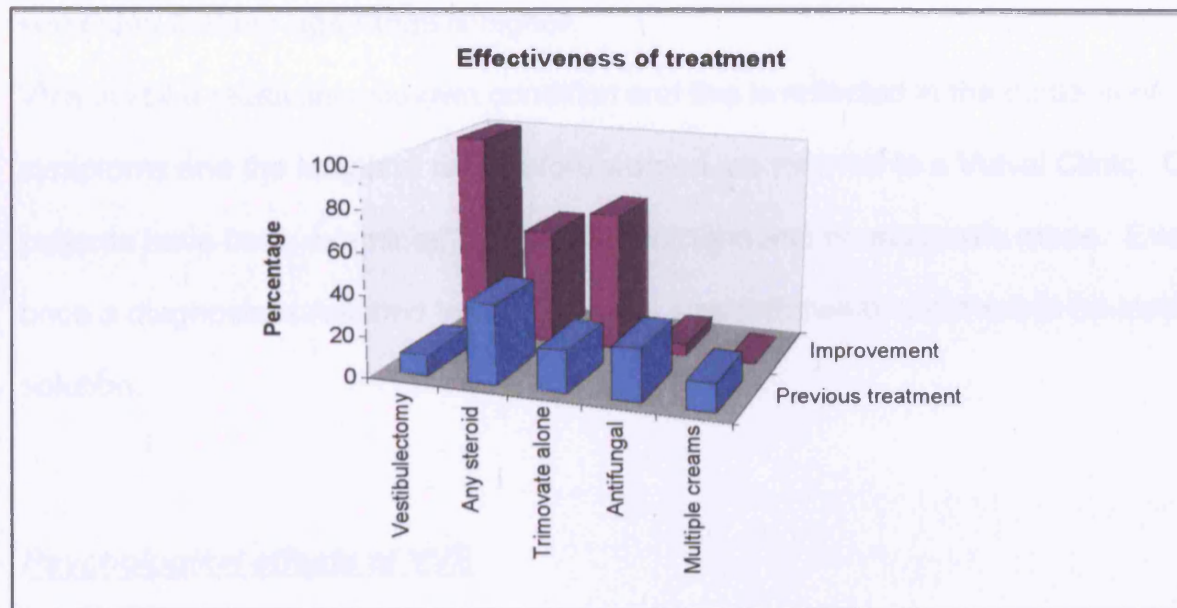
Thirteen percent reported that diet affected their symptoms with over half of these reporting symptoms worsening with alcohol. Only one found a low oxalate diet helpful, (Solomons 1991) but some improved with reduced yeast diet.

### **Previous treatment**

The cohort was asked if any treatment they had received had improved their symptoms. Considering the high incidence of misdiagnosis and the lack of knowledge about this condition, it is not surprising that treatment patterns prior to referral to the Vulval Clinic were random. Thirty-eight percent of the cohort had used multiple topical creams, but only one patient had experienced relief of symptoms. There was a better response in the group who had used steroid based preparations (58%), of which 60% had improved. Those who had used Trimovate alone had a 67% response rate.

Six of the 61 patients had undergone vestibulectomy, using the surgical technique described by Marinoff (1991) and these had all improved significantly, both clinically as well as achieving maximal algometer scores, and did not require further treatment. The percentage of patients receiving specific treatment and their response is shown in Fig 3.7

Fig 3.7 Response to previous treatment



## Discussion

Epidemiological data about British women with VVS is uncommon. The results of this epidemiological survey have been compared with published data from around the world. Our prevalence of 10-15% is comparable with that reported by Goetsch (1991), although her prevalence was from a private general gynaecological clinic and not a specialist vulval clinic. The predominance of Caucasian women is also previously reported.

Our median age of 34.6 years is comparable with Goetsch's population but significantly higher than the Swedish population who were all under 26 years (Sjoberg et al 1997).

In view of the fact that some of our patients had symptoms for over a decade, it is not surprising that our age range is higher.

VVS is still a relatively unknown condition and this is reflected in the duration of symptoms and the length of time before women are referred to a Vulval Clinic. Often patients have been examined by multiple clinicians and no diagnosis made. Even once a diagnosis is reached treatment is not straightforward and there is no instant solution.

### **Psychological effects of VVS**

Often in the absence of obvious physical abnormality, the suggestion that the condition is psychosomatic or purely psychological is raised. This frequently leads to antagonism between the patient and the clinician, and can destroy the trust the woman needs to feel within the doctor patient relationship in order to manage this condition successfully. It is easy to imply that “all is in the mind” when no straightforward physical solution is available. Unfortunately this attitude may be reflected by the woman’s partner and may even be seen by the partner as an excuse to avoid physical contact. The stresses that this places on a couple are great and this condition has been cited as contributing to the breakdown of relationships.

Studies into the psychological effects of vulval disease have been published. Often the making of a diagnosis is enough to ease some of the tension. Many of our cohort reported that they were relieved to finally have a diagnosis and to be able to put a name to the symptoms that they had been unable to explain. Stewart et al (1994)

showed that women with vulvodynia have a greater degree of psychological stress than women with other vulval pathology, but women who have vulvodynia with an identifiable cause have less stress than those with essential or dysaesthetic vulvodynia.

Jantos and White (1997) highlighted the parallelism between women with VVS and somatisation disorders, but we did not find any significant evidence of this in our cohort.

### **Quantification of symptoms**

In the literature, most authors have not asked their cohort to quantify pain. We used a visual analogue score to quantify symptoms, where a score of 0 indicates the patient is asymptomatic, and a score of 10 indicates the patient cannot imagine the symptom becoming worse. By asking the cohort to do this for all five categories of vulval burning, dyspareunia, pruritus, discharge and difficulty in tampon use we were able to establish the significance they attached to each symptom. Jantos and White (1997) previously used a similar method to look at dyspareunia within vulval pain syndromes and found their cohort had a mean pain rating of 8.5. Our initial mean rating for dyspareunia was 7.6 and was our highest ranked symptom. Less importance was attributed to pruritus and discharge. This is not surprising, as although pruritus is a prominent feature of other vulval pathologies it is not a classical symptom of VVS.

## **Aetiological Factors**

### **Hormonal**

Use of the combined oral contraceptive pill (cOCP) has been implicated in the aetiology of VVS. Sjoberg and Nylander (1997) found their patients with VVS had used the cOCP for a significantly longer time than a control group, and Bazin et al (1994) found a greatly increased relative risk with early use of the cOCP. Other studies have not looked at this in depth.

In our study the combined oral contraceptive pill was the most widely used method of contraception, but equivalent numbers were no longer using any contraception, due to their sexual inactivity. About three-quarters of the cohort had used the oral contraceptive pill in the past.

Previous studies have not commented on change in symptoms with different contraceptive use. We found the most common change was that symptoms had worsened with the oral contraceptive pill (18%) and of those who thought their symptoms had started with a change of contraception most commonly it was when they had started the pill.

We know that the combined oral contraceptive pill suppresses measured endogenous oestrogen (Gilmer et al 1980). We also know that breast-feeding women are hypo oestrogenised. The group of women whose symptoms improve when they stop the contraceptive pill or breast feeding gives rise to the theory of a possible hormonal aetiology of Vulval Vestibulitis Syndrome. This has not been researched in the literature but is investigated and discussed further in chapter 5.

VVS is assumed to be a condition that affects younger women, however 14% of our cohort were post menopausal, having had symptoms for up to 20 years, although all had been premenopausal when the condition started. It has been reported that early menarche may be associated with an increased relative risk of developing VVS (Bazin et al 1994), but with our median age of menarche being 13 our cohort does not fall into this category. This characteristic is in concordance with previous studies (Peckham et al 1986), as was cycle length and the 25% of patients suffering dysmenorrhoea. Half of our patients thought that their symptoms were cyclical but there was no correlation with a particular time of cycle. Schover et al (1992) commented specifically on pain in relation to the menstrual cycle, having found their cohort experienced exacerbation of symptoms in the late luteal phase. We found that only 16% of our population thought that pain was worse at one point during their menstrual cycle but there was no correlation with phases of the cycle.

Wide variation was found with reported tampon use. Peckham et al (1986) quoted that all of his patients used tampons, whereas Goetsch (1991) found that three quarters of her cohort did not use tampons. In our study just under half regularly used tampons (45%), although a few in this group found them uncomfortable, but not painful.

The incidence of post partum vestibulitis (Goetsch 1991, Woodruff and Parmley 1983) is reported as greater than found in our study. Vaginal delivery was not a precipitating factor frequently found in our population. (Fig 3.6) As previously mentioned, mode of

delivery, and therefore perineal trauma do not appear to be relevant, but other studies have not investigated this. Breast-feeding appeared to be more important, as a proportion of the women who developed symptoms post delivery improved when they stopped breast-feeding.

### Sexual

The majority of our cohort could identify a precipitating factor. Goetsch (1991) postulated the existence of primary and secondary vestibulitis. Primary vestibulitis is dyspareunia experienced from first coitus. Secondary vestibulitis is when symptoms develop later, having previously had painfree penetrative intercourse. If these definitions are applied to our population, the proportion of the cohort who had symptoms from first intercourse is low at 8%. This conflicts evidence from Canada when a study found 43.9% of their surveyed cohort experienced dyspareunia compatible with Vulval Vestibulitis Syndrome, at first coitus (Bazin et al 1994). The same study demonstrated an increased relative risk with early sexual activity before 16 years of age. The median onset of first intercourse in our group was 18, and only 6 patients in our group were less than 16 years old at first coitus, and so data from our cohort does not support this theory. As previously reported, frequency of sexual intercourse falls dramatically with onset of this condition, and with half of our cohort abstaining totally, this shows what an impact this condition has on women's lives.



Peckham et al (1986) reported that 60% of his cohort had new partners within 6 months of developing symptoms, but in our cohort only 13 % associated symptoms starting with a new partner. However the majority of our patients had not had symptoms with a previous partner, but as the median length of current relationship was 7.6 years and median duration of symptoms 4.5 years this is not surprising.

The most common precipitating event in our cohort was a health event or infection. A large proportion attributed commencement of symptoms to an episode of fungal infection, but these were not microbiologically proven episodes. It is widely assumed, both by the public and the medical profession that any vulval symptoms are caused by candida infection, and so it is not possible to distinguish between genuine, possibly causative infections and misdiagnosis. This problem also affects the initial treatment given prior to referral.

### Previous treatment

When questioned about previous treatment again the variation in approach was highlighted, as was the difficulty in achieving successful treatment results. The minimal improvement in the group who had multiple topical creams shows that unless there is superimposed fungal infection, anti fungal treatment has no place in treatment of VVS. This contradicts evidence suggesting it should be first line treatment (Morrison et al 1996).

As mentioned previously, many vulval patients report improvement having only achieved a diagnosis. If this is true, then the initial physical therapy is irrelevant, as a

proportion would have improved without physical treatment. Equally, the placebo effect in vulval disease is poorly understood. Some patients find relief with any topical cream, and it may be the emollient vehicle that is effective and not the active ingredients.

The total success of the patients in our cohort who had surgery is higher than the recognised figure (Marinoff and Turner 1991) but the numbers in our group were small and may represent patient selection. Surgery is not the first line treatment of Vulval Vestibulitis Syndrome in this country, as it is elsewhere in the world, particularly in parts of the United States, but it appears to be successful. Why removing this skin that has no histopathological abnormality works, however remains unknown. In our unit we only operate on patients who have “failed” all medical treatment and in whom all other causes of the vulval symptoms have been excluded and so represent women with more severe or resistant disease

### Other factors

Diet affecting symptoms has been postulated and low oxalate diets recommended as treatment in the past (Solomons et al 1991), but only 13% of our cohort found any relationship between diet and symptoms. In those who did, alcohol was the most common factor exacerbating symptoms. Only one patient improved on the low oxalate diet.

Genetic factors have also been suggested and high rates of affected female relatives have been expressed (Goetsch 1991), but we found only 2 patients who had female relatives with similar difficulties.

## **Conclusion**

The assumption that VVS, although a physical problem, has some psychological component is reasonable, but it is difficult to distinguish whether this causes symptomatology or is a result of it. Many patients have come to clinic having been given multiple diagnoses and treatments by different clinicians and there is an obvious underlying fear of not being believed. Equally many of these women have had symptoms for such a long period of time that they can no longer remember what is “normal” and are beginning to question their own judgement.

Our data from British patients appear comparable with other studies elsewhere in the world. There does not appear to be a specific pattern developing to characterise women with this condition, apart from the symptoms that they all suffer. Obviously as the aetiology remains unclear, this presents continuing difficulty for the clinician in establishing the best method of treatment for their patient.

Many aetiological factors have been investigated and excluded. Our epidemiological data show that many factors that appear to affect this condition are hormonal. With the exception of the few studies mentioned, these factors have been largely ignored and there has not been formal investigation into hormonal aetiology.

This issue is addressed in chapter 5.

## **CHAPTER FOUR - IS VULVAL VESTIBULITIS SYNDROME AN INFLAMMATORY CONDITION?**

### **Introduction**

There is a growing belief that if VVS is not an infection, it is the result of an inflammatory process. Histopathological studies have failed to recognise specific features unique to this condition, but chronic inflammatory infiltrates are consistently seen (Pyka et al 1988). The histopathological appearance of a normal vestibule, stained with Haematoxylin and Eosin is shown in Fig 1.2.

Inflammation is the response of a vascularised tissue to local injury. Inflammation causes vascular damage, leading to hyperaemia, and this would account for the vestibular erythema seen in VVS.

Pro-inflammatory mediators, including cytokines, regulate inflammation.

Cytokines are soluble proteins, which act as chemical communicators between cells but not as effector molecules within their own right.

Tumour Necrosis Factor (TNF)- $\alpha$  is a cytokine secreted by activated monocytes and macrophages and is a potent endocrine and paracrine mediator of inflammation. It interacts with other inflammatory mediators such as histamine and substance P to

promote neurogenic inflammation. It has a particular effect on Interleukin-1 $\beta$  (IL-1 $\beta$ ) by providing a positive feedback loop to promote increased production of IL-1 $\beta$  and release of other cytokines.

IL-1 cytokines are polypeptides that are centrally involved in the genesis and maintenance of the inflammatory response, their major physiological source being the activated macrophage. They cause prostaglandin release via action on fibroblasts and can act on endothelial cells to promote TNF $\alpha$  release. IL-1 $\alpha$  and 1 $\beta$ , along with TNF $\alpha$  are important inducers of the acute phase response of inflammation (Sims and Dower 1994). TNF receptors are present on nearly all cell types except erythrocytes and resting T cells and IL-1 receptors are found on all nucleated cells (Thompson 1994).

If VVS is an inflammatory condition, my hypothesis is that tissue from patients with VVS should have greater expression of inflammatory markers than tissue from normal controls.

Only one previous study has investigated TNF $\alpha$  in VVS patients, but used ELISA techniques on homogenised tissue (Foster and Hasday 1997). My contribution is to demonstrate TNF $\alpha$ , IL-1 $\alpha$  and 1 $\beta$  using immunohistochemistry, which allows the spatial relationship of proteins to be assessed maintaining tissue morphology. Expression of these cytokines in women with VVS could then be compared to expression in normal vestibular tissue.

## **Immunohistochemistry: the Theory**

Immunohistochemistry is the process of staining tissue with antibodies to identify a particular protein expression, and to demonstrate the positioning of such proteins within a tissue. The protein that is being investigated has antigenic properties and expresses epitopes, to which the antibody binds. A visible marker is attached to the antibody, thereby making the protein visible under microscopic analysis.

Antibodies are proteins produced in response to 'foreign' material that acts as an antigen, by B-lymphocytes in the spleen. Monoclonal antibodies are derived from a single B-cell and are therefore specific in their origin. This property is used in immunohistochemistry to obtain specificity within a reaction and thus allow identification of expression of a particular protein.

Polyclonal antibodies are derived from several B cell lines and so are not specific.

Both monoclonal and polyclonal antibodies may be used in immunohistochemistry but, as the process is usually trying to identify a particular protein, monoclonal antibodies are used more frequently, if they are available, as they are more specific.

### **Production of monoclonal antibodies**

#### **1) Immunisation**

A particular protein, which acts as an immunogen, is injected into a small animal such as a mouse. The mouse then mounts an immune response and produces antibodies to the injected protein. The B-cells are then removed from the mouse spleen or lymph

node. To obtain the monoclonal antibody it is necessary to isolate the specific B-cell from the other B-cells. This is performed in tissue culture where proteases dissociate cell clusters in the medium into single cells, which can then be isolated.

## 2) Fusion and selection

The B-cell is capable of cloning itself, but will have a finite lifespan. In order to prolong cell life and promote cell division the B-cell is fused with a myeloma cell to form a hybridoma. This then replicates at a greater rate and so will produce a large number of antibodies.

## 3) Screening

To obtain the desired antibody, screening enables selection of the specific antibody and non-specific antibody growth is discarded.

## 4) Characterisation

This involves further analysis of the antibody for specificity and also analyses reactivity and cross-reactivity. Included in this process is assay restriction, which involves profiling the antibody in different assay systems. This is important for the antibody's potential as a diagnostic reagent, as some monoclonal antibodies will perform well in some systems but not in others.

Once the specific antibody is made, bulk production can then be achieved using surface expanded tissue culture flasks or hollow fibre systems (Nelson et al 2000).

There are a variety of techniques and methods employed in immunohistochemistry. The following gives a brief account of the methods used in this project.

### Indirect 2 Step Method

In this method the primary antibody reacts with the antigen in the tissue. The secondary antibody is labelled with the visible enzyme, and the secondary antibody binds to the primary antibody. The secondary antibody must be directed against the species that the primary antibody is derived from. This process enables several different antibodies to bind with different epitopes on the primary antibody, which means that more markers will be attached at the site of the antigen. Therefore, this procedure will give better results with increased intensity of staining, and will be more specific.

The problem with this is that there is a greater chance of cross-reactivity with this method, which will result in increased non-specific background staining. This is overcome by using secondary serum from the same species as the tissue under investigation (i.e. human) which will 'mop up' non-specific endogeneous immunoglobulin.

This method was used throughout this project.

### Avidin-Biotin Method

This method uses the high affinity of avidin or streptavidin for biotin therefore making it a more sensitive method. Biotinylation is the process by which biotin is covalently



attached to the antibody. The secondary antibody is biotinylated and horseradish peroxidase is used as an enzyme label. This method has been found to be more sensitive than other immunohistochemical methods and has excellent results on fixed, paraffin embedded tissue.

### **Use of enzymes in immunohistochemistry**

Immunohistochemical methods use enzyme-substrate reactions to produce coloured end-products which are visible under the microscope from colourless chromogens.

Enzymes used in immunohistochemistry should have the following properties:

- 1) The bound enzyme should be stable in solution.
- 2) The enzyme should be available in a highly purified form.
- 3) Conjugation or non-covalent binding should not abolish enzyme activity.
- 4) Enzyme activity should interfere minimally with specific antigen related staining.
- 5) Products of reactions should be readily detectable and stable.

### **Enzyme labels - Horseradish Peroxidase and DAB**

These reagents are suitable for labelling as the haeme group within the horseradish peroxidase reacts with hydrogen peroxide to form a complex, which then decomposes to water and atomic oxygen. This reaction can then oxidize an electron donor, such as 3,3'-diaminobenzidine (DAB), which then converts from a colourless compound to a coloured (brown) product and therefore can be used as a chromogen.

### **Establishing correct dilutions**

In order to obtain optimal results, immunohistochemistry should produce staining of sufficient intensity to be clearly visible for the protein under investigation, with minimal background staining of the surrounding tissue.

Antibody titre is defined as the highest dilution of an antiserum that results in optimal specific staining with least amount of background staining.

The highest dilution of a substance is governed by the affinity of the antibody. A high affinity antibody is likely to react faster with an antigen, to produce a more intense stain in a shorter incubation period. Low affinity antibodies may need a longer incubation period to achieve the same intensity of stain. Long incubation periods should be performed in a humidity chamber to prevent evaporation and drying out of tissue sections. Equally, higher dilutions can be used with longer incubation times. Incubation temperatures may also vary, but commonly are at room temperature or 4°C. Experimental data with small quantities of different dilutions are often necessary to establish optimum dilution prior to proceeding with the main staining experiment.

### **Antigen Retrieval**

When using tissue in paraffin-embedded sections, the antigens sometimes need to be retrieved from the tissue in order for them to be available to bind freely with the antibody that is being used for the investigation. This may be performed using microwaving or protease digestion. Therefore, when designing an

immunohistochemical protocol on paraffin-embedded sections, often all antigen retrieval methods are tried during trial experiments, and compared to no antigen retrieval, to see which method gives the best results, before the study experiments are performed.

In this study, microwaving was performed in 10mM sodium citrate buffer (pH 6.0) in a microwave at full power for 5 minute bursts twice. Protease digestion used 125µg/ml PBS, Bacterial Type XXIV protease (P-8038, Sigma) for 7.5 minutes at 37°C.

### **Controls**

Controls are vital for validation of immunohistochemistry and should be included in any staining procedure.

#### **Positive controls**

Positive controls were used throughout this project, appropriate for each target tissue.

Tissue known to contain the target antibody, was used for each experiment.

For oestrogen receptor expression, breast cancer tissue with known oestrogen receptor positive status was used. For cytokine expression, breast or ovarian cancer tissue was used

#### **Negative controls**

Negative controls were used in each experiment. These were the same tissue specimens used for the positive control, but addition of the primary antibody was omitted and replaced by the buffer being used.

### **Background Staining**

Background staining is the result of endogeneous enzyme activity. This results from decomposition of hydrogen peroxide by haeme proteins such as haemoglobin contained within tissue samples. This can be overcome by addition of hydrogen peroxide during the staining process to absorb any excess activity.

## **STUDY**

### **Methods and Materials**

Following ethical approval, 35 tissue samples from the vestibule of the vulva were obtained from patients who fulfilled Friedrich's criteria of VVS. This was performed either at vestibulectomy in patients undergoing surgical treatment, or from outpatient biopsy using a 4mm Stiefel punch.

Some of the women had previously had medical treatment including topical steroids and topical oestrogen, but none of the women were using medical treatment at the time the biopsies were taken. Six of the group were currently using the OCP and one was postmenopausal.

Following approval from the local Ethics Committee, sixteen samples of normal vestibular tissue were obtained from women undergoing unrelated gynaecological surgery. All of these women were asymptomatic and not taking hormonal contraception.

All of the control group and all but one of the study group were premenopausal. In both groups all the women were Caucasian and the controls were selected to age match the study group.

All tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Five micrometre sections were cut and placed on 3-aminopropyltriethoxysilane-coated glass slides.

The slides were stained with haematoxylin and eosin and were reviewed by a pathologist to exclude other pathology.

### **Specific Immunohistochemical staining techniques**

#### **TNF $\alpha$**

Immunohistochemical staining for TNF $\alpha$  was performed using a standard streptavidin biotin technique with a commercially available monoclonal antibody to TNF $\alpha$ .

Trial experiments were performed using different dilutions of antibody to establish the correct dilution of primary antibody to obtain optimal staining. Dilutions varied between neat application of the antibody and 1:50. Optimal staining with positive results but no background staining was reached using a dilution of 1:5.

Experiments for antigen retrieval were performed using microwaving and protease digestion, and varying incubation of either 1 hour or overnight was tried. Different temperatures of either 4°C or room temperature were also tried for the incubation period. The final full protocol is shown in Appendix 4.1.

Positive control tissue was breast cancer, and a negative control where the primary antibody was omitted, was used for each immunohistochemical experiment. Staining was repeated four times to ensure staining reproducibility.

Positive expression of TNF- $\alpha$  stained brown; slides were counterstained with haematoxylin. Negative expression stained blue.

### IL-1 $\alpha$

Immunohistochemical staining for IL1 $\alpha$  was performed using a standard streptavidin biotin technique, using a commercially available antibody to IL1 $\alpha$ .

Trial experiments were performed using different dilutions of antibody to establish the correct dilution of primary antibody to obtain optimum staining. Dilutions varied between 1:5 and 1:100. Optimal staining with positive results but no background staining was reached using a dilution of 1:20.

Experiments for antigen retrieval were performed using microwaving and protease digestion, and varying incubation of either 1 hour or overnight was tried. Different temperatures of either 4°C or room temperature were also tried for the incubation period. The final full protocol is shown in Appendix 4.2.

Positive control tissue was ovarian cancer, and a negative control where the primary antibody was omitted, was used for each immunohistochemical experiment. Staining was repeated four times to ensure staining reproducibility.

Positive expression of IL-1 $\alpha$  stained brown; slides were counterstained with haematoxylin. Negative expression stained blue.

### IL1 $\beta$

Immunohistochemical staining for IL1 $\beta$  was performed using a standard streptavidin biotin technique, using a commercially available antibody to IL1 $\beta$ .

Trial experiments were performed using different dilutions of antibody to establish the correct dilution of primary antibody to obtain optimal staining. Dilutions varied between 1:50 and 1:500. Optimal staining with positive results but no background staining was reached using a dilution of 1:100.

Experiments for antigen retrieval were performed using microwaving and protease digestion, and varying incubation of either 1 hour or overnight was tried. Different temperatures of either 4°C or room temperature were also tried for the incubation period. The final full protocol is shown in Appendix 4.3.

Control tissue was ovarian cancer, and a negative control where the primary antibody was omitted, was used for each immunohistochemical experiment. Staining was repeated four times to ensure staining reproducibility.

Positive expression of IL-1 $\beta$  stained brown; slides were counterstained with haematoxylin. Negative expression stained blue.



### **Analysis of staining**

The sections were analysed by light microscopy (x200 magnification: x10 eyepiece, x20 objective) by two independent observers who were unaware whether the section was from a control or a VVS patient. Less than 5% discrepancy was found in most of the scores, but in sections where the result was inconsistent, the slide was re-examined by both observers and agreement reached.

As the samples were small the whole section, i.e. the dermis and epidermis, was analysed.

### **Scoring Systems**

#### **TNF $\alpha$**

All the samples that showed expression of TNF- $\alpha$ , stained positive in more than 50% of the whole sample (i.e. dermis and epidermis), therefore the samples were said to be positive for TNF $\alpha$  expression if they showed any positive staining (i.e. >50%). A negative result, i.e. not expressing TNF $\alpha$ , was only recorded if the whole sample was negative.

#### **IL-1 $\alpha$**

The dermis and epidermis of the whole section were analysed. Nuclear and cytoplasmic staining was recorded separately. Degree of expression was recorded as positive if the whole section expressed IL-1 $\alpha$ , mainly positive if the positive areas were

much greater than the negative areas (i.e. >60%), negative if the whole section did not express IL1 $\alpha$  and mainly negative if the negative areas were far greater than the positive areas (i.e. >60%). Intensity of staining was not formally assessed, as all sections were similar.

### IL1 $\beta$

Nuclear and cytoplasmic staining of the whole sample, i.e. dermis and epidermis were recorded. Degree of expression was recorded as positive if the whole section expressed IL-1 $\beta$ , mainly positive if the positive areas were much greater than the negative areas (i.e. > 60%), negative if the whole section did not express IL-1 $\beta$  and mainly negative if the negative areas were far greater than the positive areas (i.e. >60%).

Intensity of staining was not formally assessed, as all sections were similar, although the intensity was reduced overall in comparison to the staining seen in the sections expressing IL-1 $\alpha$ , probably because it was a polyclonal antibody.

### **Statistics**

Statistical analysis was performed using the Minitab Statistics package for Windows.

## **Results**

When the sections were analysed under the microscope, it became obvious that the differences between the sample from the controls and the VVS patients could be seen only in the epidermis. Therefore the results below are for cytokine expression in the epidermis of the vestibular tissues.

### **TNF $\alpha$**

Table 4.1 shows the comparison of TNF- $\alpha$  expression between the two groups.

Table 4.2 shows the results of TNF $\alpha$  expression in the sections.

Only 11% (n=4) of the vestibulitis cohort stained positive for expression of TNF- $\alpha$ , with 89% (n=31) showing no expression of TNF- $\alpha$  in any layer of the specimen. However 56% (n=9) of the control samples expressed TNF- $\alpha$  with the minority of the cohort (44%, n=7) showing no expression of TNF- $\alpha$ . The samples from the patients with VVS showed less expression of TNF $\alpha$  than the control tissue, with the majority of the VVS not expressing TNF $\alpha$  at all.

Using a Chi-Squared test, these results show a statistically significant difference (p=0.001).

Table 4.1 Results: Summary of TNF- $\alpha$  expression

<b><u>STAINING</u></b>	<b><u>VVS</u></b>	<b><u>CONTROL</u></b>
POSITIVE	11% (n=4)	56% (n=9)
NEGATIVE	89% (n=31)	44% (n=7)

Table 4.2 TNF  $\alpha$  results: VVS and controls

SUBJECT	SAMPLE	RESULT	CONTROL	SAMPLE	RESULT
975954	P	Negative	1	P	Positive
976017	V	Negative	2	P	Positive
921212	V	Negative	3	P	Negative
94419	V	Positive	4	P	Negative
978704	P	Negative	5	P	Positive
971668	V	Negative	6	P	Negative
963371	V	Negative	7	P	Positive
974024	V	Negative	8	P	Negative
946844	V	Negative	9	P	Positive
94861	V	Negative	12	P	Positive
943709	V	Positive	13	P	Positive
956595	V	Negative	14	P	Negative
972740	V	Negative	17	P	Positive
190697	P	Negative	18	P	Positive
190697b	P	Negative	20	P	Negative
190697c	P	Negative	21	P	Negative
979632	P	Negative			
9711579	P	Negative			
943708	V	Negative			
954773	V	Negative			
916353	V	Negative			
9712093	V	Negative			
975629	P	Negative			
9713276	P	Negative			
953949	V	Negative			
973858	P	Negative			
9712432	P	Positive			
94169	V	Negative			
978868	V	Negative			
97474	V	Positive			
9610453	P	Negative			
968886	P	Negative			
95436	V	Negative			
9714579	P	Negative			
975839	V	Negative			

P = Punch biopsy

V = Vestibulectomy specimen

Sections from control and VVS tissue, expressing  $\text{TNF}\alpha$  are shown in Figs 4.1 and 4.2

Fig 4.1  $\text{TNF-}\alpha$  expression in VVS tissue

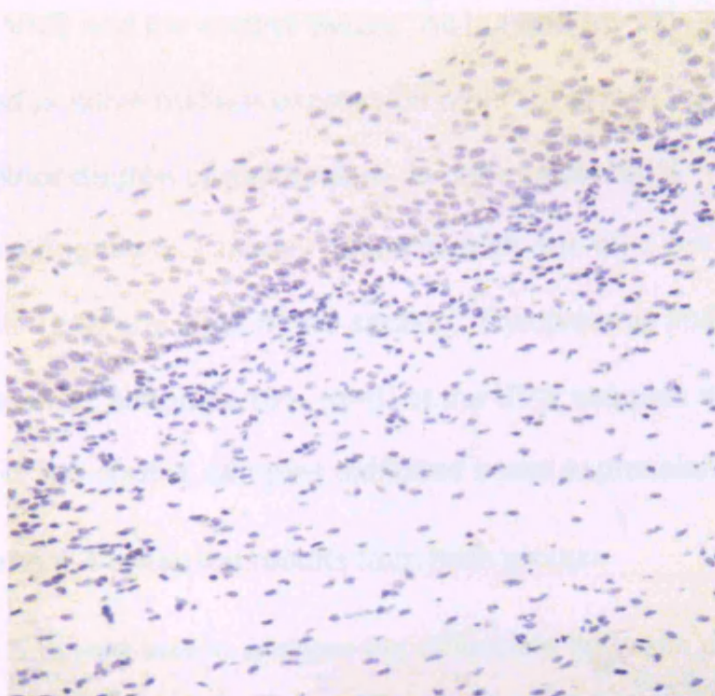
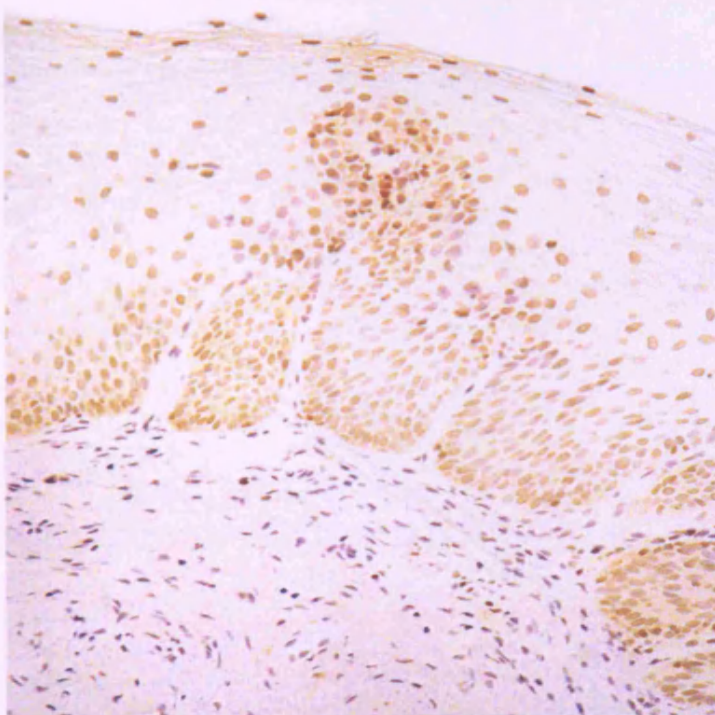


Fig 4.2  $\text{TNF-}\alpha$  expression in control tissue



### **IL-1 $\alpha$**

A marked difference was again obtained between expression of IL-1 $\alpha$  in tissue from women with VVS and the control tissue. All but one (n=15) of the control specimens demonstrated positive nuclear expression of IL-1 $\alpha$ , and in the remaining sample there was some minor degree of expression. In comparison 65% (n=22) of the VVS samples were negative, or mainly negative, with one third (n=12) of the specimens not expressing IL-1 $\alpha$  in any layer of the section. Cytoplasmic staining did not show such a marked difference, although 16% (n=6) of the VVS samples were entirely negative, whereas all of the control samples exhibited some expression.

Tables 4.3 and 4.4 show the results from both groups.

Using a Chi Squared test to analyse the difference between positive and negative staining between the VVS group and the control group, a statistically significant difference was found in nuclear staining (p=0.0005), but not in the cytoplasmic staining (p=0.2)

Table 4.3 IL-1 $\alpha$  VVS Results

<b>SUBJECT</b>	<b>SAMPLE</b>	<b>RESULT (nuclear)</b>	<b>RESULT (Cytoplasm)</b>
975954	P	Positive	Positive
976017	V	Pos + Neg	Positive
921212	V	Negative	Positive
94419	V	Negative	Positive
978704	P	Neg ,Occ Pos	Positive
971668	V	Negative	Negative
963371	V	Neg + Pos	Positive
974024	V	Negative	Positive
946844	V	Negative	Negative
94861	V	Negative	Positive
943709	V	Pos Occ Neg	Positive ++
956595	V	Pos + Neg	Positive
972740	V	Neg + Pos	Positive
190697	P	Negative	Negative
190697b	P	Negative	Positive
190697c	P	Positive	Negative
979632	P	Negative	Positive
9711579	P	Negative	Positive
943708	V	Neg,Occ pos	Positive
954773	V	Pos + Neg	Positive
916353	V	Neg, Occ Pos	Positive
9712093	V	Positive	Positive
975629	P	Neg + Pos	Positive
9713276	P	Positive	Positive
953949	V	Negative	Neg + Pos
973858	P	Pos + Neg	Positive
9712432	P	Pos + Neg	Positive
94169	V	Positive	Positive
978868	V	Neg + Pos	Pos + Neg
97474	V	Neg + Pos	Positive
9610453	P	Pos + Neg	Positive
968886	P	Positive	Negative
95436	V	Negative	Negative
9714579	P	Neg + Pos	Positive
975839	V	Neg + Pos	Positive

P= Punch Biopsy  
V= Vestibulectomy

Neg+Pos = mainly negative  
Pos+Neg = mainly positive

Table 4.4 IL-1 $\alpha$  Control Results

CONTROL	SAMPLE	RESULT (nuclear)	RESULT (cytoplasm)
1	P	Pos, Occ Neg	Neg + Pos
2	P	Pos + Neg	Positive
3	P	Pos, Occ Neg	Positive
4	P	Pos + Neg	Pos + Neg
5	P	Pos + Neg	Positive
6	P	Pos + Neg	Positive
7	P	Pos + Neg	Positive
8	P	Neg + Pos	Positive
9	P	Pos, Occ Neg	Positive
12	P	Pos + Neg	Positive
13	P	Pos, Occ Neg	Positive
14	P	Pos, Occ Neg	Positive
17	P	Pos + Neg	Positive
18	P	Pos + Neg	Positive
20	P	Pos + Neg	Positive
21	P	Pos + Neg	Positive

P = Punch biopsy

V = Vestibulectomy specimen

Neg+Pos = mainly negative

Pos+Neg = mainly positive

Table 4.5 shows the comparison of IL-1 $\alpha$  expression between the two groups

Sections from control and VVS tissue, expressing IL-1 $\alpha$  are shown in Figs 4.3 and 4.4.



Fig 4.3 IL-1<sub>α</sub> expression in VVS tissue

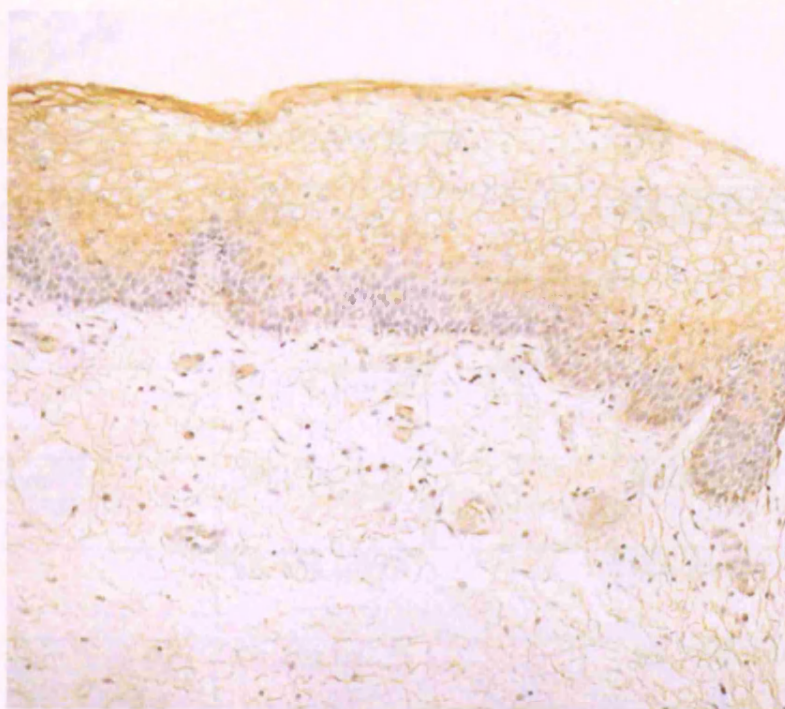


Fig 4.4 IL-1<sub>α</sub> expression in control tissue

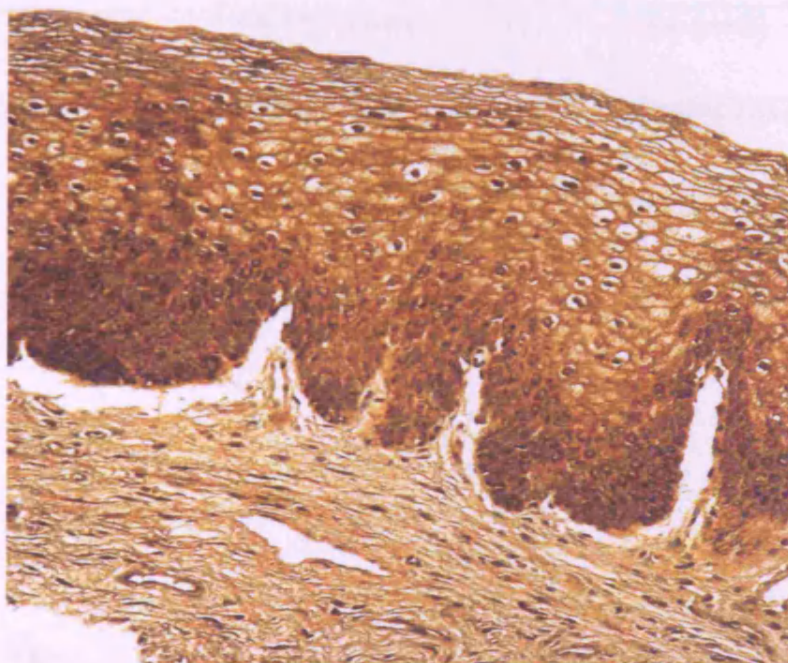


Table 4.5 Results: Summary of IL-1 $\alpha$  expression

<b><u>STAINING</u></b>	<b><u>VVS</u></b>	<b><u>CONTROL</u></b>
<b><u>NUCLEAR</u></b>		
Negative	35.5% (n=12)	0% (n=0)
Mainly negative	29% (n=10)	6.25% (n=1)
Positive	16.1% (n=6)	0% (n=0)
Mainly positive	19.4% (n=7)	93.75% (n=15)
<b>CYTOPLASMIC</b>		
Negative	16.1% (n=6)	0% (n=0)
Mainly negative	3.2% (n=1)	6.25% (n=1)
Positive	77.5% (n=27)	87.5% (n=14)
Mainly positive	3.2% (n=1)	6.25% (n=1)

### **IL-1 $\beta$**

There was little nuclear expression of IL-1 $\beta$  in both the VVS and control samples.

Results are shown in Tables 4.6 and 4.7

All but one (97%, n=34) of the VVS specimens did not show any nuclear expression of IL-1 $\beta$  in any layer of the section, and 94% (n=15) of the control tissue was negative, or mainly negative. However positive staining was seen in the cytoplasm of all the controls and 87% of the VVS samples. Using a Chi Squared test, there was no statistically significant difference between the nuclear staining (p=0.3), or the cytoplasmic staining (p=0.29).

Table 4.6 IL-1 $\beta$  Control Results

CONTROL	SAMPLE	RESULT (nuclear)	RESULT (cytoplasm)
1	P	Neg + Pos	Positive
2	P	Negative	Positive
3	P	Negative	Positive
4	P	Negative	Positive
5	P	Neg + Pos	Positive
6	P	Neg + Pos	Positive
7	P	Negative	Positive
8	P	Negative	Positive
9	P	Negative	Positive
12	P	Neg + Pos	Positive
13	P	Negative	Positive
14	P	Neg + Pos	Positive
17	P	Neg + Pos	Positive
18	P	Neg + Pos	Positive
20	P	Pos + Neg	Positive
21	P	Negative	Positive

P= punch biopsy

Neg+Pos = mainly negative  
Pos+Neg = mainly positive

Table 4.7 IL-1 $\beta$  VVS Results

<b>SUBJECT</b>	<b>SAMPLE</b>	<b>RESULT (nuclear)</b>	<b>RESULT (cytoplasm)</b>
975954	P	Negative	Neg + Pos
976017	V	Negative	Negative
921212	V	Neg, Occ Pos	Positive
94419	V	Negative	Positive
978704	P	Negative	Positive
971668	V	Negative	Positive
963371	V	Negative	Positive
974024	V	Negative	Neg + Pos
946844	V	Negative	Neg + Pos
94861	V	Negative	Positive
943709	V	Negative	Pos + Neg
956595	V	Negative	Positive
972740	V	Negative	Positive
961552	V	Negative	Positive
190697	P	Negative	Pos + Neg
190697b	P	Negative	Positive
190697c	P	Negative	Pos + Neg
979632	P	Negative	Positive
9711579	P	Negative	Positive
943708	V	Negative	Positive
954773	V	Negative	Positive
916353	V	Negative	Positive
9712093	V	Negative	Positive
975629	P	Negative	Positive
9713276	P	Negative	Positive
953949	V	Negative	Positive
973858	P	Negative	Positive
9712432	P	Negative	Positive
94169	V	Negative	Positive
978868	V	Negative	Positive
97474	V	Negative	Positive
9610453	P	Negative	Positive
968886	P	Negative	Positive
95436	V	Negative	Positive
9714579	P	Negative	Positive
975839	V	Negative	Positive

P = Punch biopsy  
V = Vestibulectomy specimen

Neg+Pos = mainly negative  
Pos+Neg = mainly positive

Table 4.8 Results: Summary of IL-1 $\beta$  expression

<b><u>STAINING</u></b>	<b><u>VVS</u></b>	<b><u>CONTROL</u></b>
<b><u>NUCLEAR</u></b>		
Negative	97% (n=34)	50% (n=8)
Mainly negative	3% (n=1)	44% (n=7)
Positive	0% (n=0)	0% (n=0)
Mainly positive	0% (n=0)	6% (n=1)
<b><u>CYTOPLASMIC</u></b>		
Negative	3% (n=1)	0% (n=0)
Mainly negative	10% (n=3)	0% (n=0)
Positive	77% (n=28)	0% (n=0)
Mainly positive	10% (n=3)	100% (n=16)

Table 4.8 shows the comparison of IL-1 $\beta$  expression between the two groups.

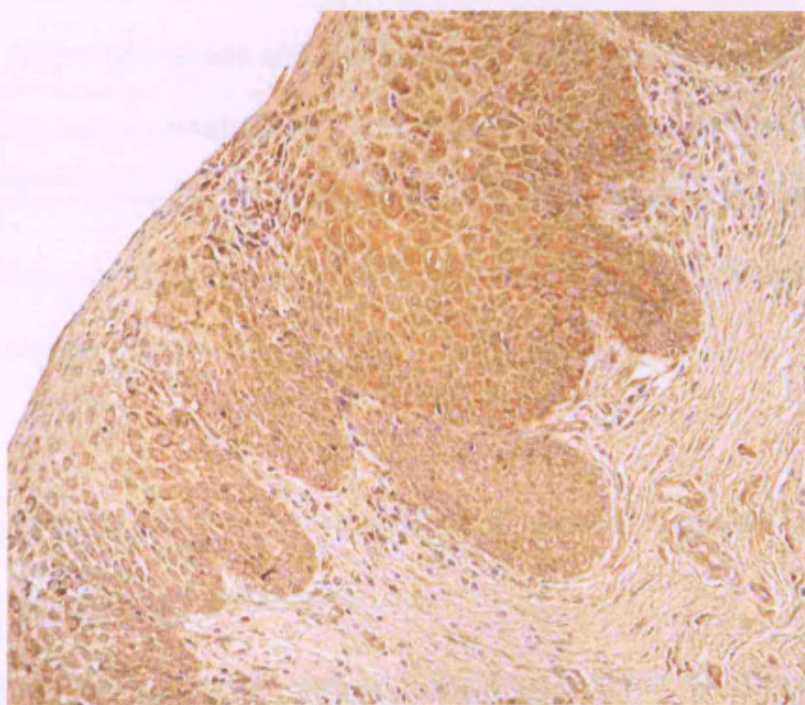
Sections from control and VVS tissue, expressing IL-1 $\beta$  are shown in Figs 4.5 and 4.6.



Fig 4.5 IL-1<sub>α</sub> expression in VVS tissue



Fig 4.6 IL-1<sub>α</sub> expression in control tissue



## **Discussion**

From these results it would appear that VVS may not be an inflammatory condition.

IL-1 and TNF $\alpha$  expression in vulval tissue in women with VVS has only been previously described in one study. Foster and Hasday (1997) performed ELISA on vulval tissue homogenates from 12 women with VVS and 10 normal controls, undergoing posterior repair, and found a 1.8 fold increased expression of TNF- $\alpha$  and 2.3 fold increased expression of IL-1 $\beta$  in women with VVS compared to an asymptomatic control group. Statistical analysis showed that the results were approaching significance ( $p=0.07$ ). However, IL-1 $\alpha$  was not examined and my study is the first study to do so.

Of interest, Foster's study found a significant paradox: when different areas of the vulva were analysed, the anatomical areas with the highest hyperalgesia expressed 2.2 fold less TNF $\alpha$  than areas elsewhere on the vulva that were not as symptomatic. Also when analysed by anatomical site, the difference between the VVS patients and controls was not significant. This led to the suggestion that cytokines were not likely to be the final common pathway, but that they acted on the vestibule secondarily through the release of neurokinines.

In order to try and explain this phenomenon it is necessary to examine other mediators of inflammatory processes.

A previous study has examined Natural Killer (NK) cell activity in women with VVS compared to a control group. NK cells secrete cytokines, including TNF $\alpha$ , and the

group found that VVS patients had significantly less NK activity (with some samples showing no activity at all), compared to the controls (Masterson et al 1996). The study suggested that NK activity could be increased in response to stimulation with IL2 or interferon but NK activity still remained significantly impaired compared with the controls.

This lack of NK activity could lead to decreased TNF $\alpha$  and IL-1 expression, as seen in our results.

Lack of TNF $\alpha$  expression has been reported in other gynaecological pathology. For example, a study looking at TNF $\alpha$  expression in normal and abnormal cervix uteri found that the cytokine was expressed in normal tissue, but was absent in 20/23 low-grade cervical intraepithelial neoplasia (CIN) specimens and 12/18 high grade CIN. This study suggested that the nature of the environment within the epithelium might act to limit effective immune response (Mota et al 1999).

It has recently been shown that women with VVS have an increased presence of the homozygous form of allele 2 of Interleukin 1 (IL-1) receptor antagonist, which is known to be a naturally occurring down regulator of the proinflammatory immune response (Jeremias et al 2000).

Regulation of the inflammatory response at skin and mucosal level is a function of interactions between inflammatory cells, cytokines and neurokinines. The individual response to TNF $\alpha$  depends on tissue levels, duration of exposure and presence of



synergistic cytokines such as IL-1 $\beta$  (Tracey 1994). If other factors involved in this response are down regulated, this may affect the expression of TNF $\alpha$ , and other inflammatory mediators may be of greater importance in the pathogenesis of VVS. This explanation may account for our findings that women with VVS appear to express less TNF $\alpha$  than the control group.

Other groups have found evidence that VVS is not inflammatory. Although Pyka et al (1988) showed a chronic inflammatory infiltrate in biopsies from women with VVS, they did not use vestibular tissue from asymptomatic women as a control. However, Lundquist et al (1997) found no difference in histomorphologic features when vestibular tissue from healthy control patients was compared with vestibular tissue from patients with VVS.

A year later, immunohistochemical analysis of the neuroendocrine cells of the minor vestibular glands found the degree of inflammation in vestibular tissue in women with and without VVS was not significantly different (Slone et al 1998).

More recently Bohm-Starke et al (2001) compared expression of inflammatory mediators cyclooxygenase 2 and inducible nitric oxide synthase in vestibular tissue from 10 women with VVS to 10 healthy controls. Both groups showed low expression and there was no difference observed between the groups. Their results indicated no

active inflammation and they implied that topical corticosteroids had no place in the treatment of VVS.

They suggested that their results indicate that the pathophysiological mechanism in VVS differs from that of chronic inflammatory diseases, and that the allodynia and erythema in the mucosa may be explained by a neuronal mechanism.

It has been proposed that unmyelinated C nociceptor fibres are activated by mediators of inflammation, including serotonin, and that protracted activation of the nociceptors sensitises "wide dynamic" neurones which produces an abnormal response to light touch mechanoreceptors with central sensation of pain (Cox 1995).

We know that from recent studies there appears to be an increase in neural fibres in the vestibule in women with VVS:

Westrom et al (1998) evaluated nerve fibre density in women with VVS. Forty-seven patients were assessed and compared to 6 controls, 4 being post mortem samples. They found increased nerve fibre proliferation in the samples from women with VVS, and suggested this would provide a morphological basis for enhanced neuronal firing, as suggested in the pain loop theory. Again the comparison was made with interstitial cystitis, where similar results were found (Christmas et al 1990).

These findings have been confirmed, showing a significant increase in intraepithelial innervation in women with VVS (Bohm-Starke 1998), suggesting focus should be on

the change in the peripheral innervation as a possible explanation for the symptoms of VVS. The authors assumed these free nerve endings were nociceptors transmitting noxious stimuli, resulting in pain. This assumption was later proved correct when the nerve fibres were neurochemically characterised. (Bohm-Starke 1999)

It had previously been shown that the vulval vestibule is rich in neuroendocrine cells and that intraepithelial axons are closely applied to these serotonin containing neuroendocrine cells. This close association and the presence of substance P immunoreactivity in local nerves had been suggested to play a role in the symptoms of VVS (Warner et al 1996).

This argument is logical in that we are aware that Substance P causes neurogenic inflammation, and there have been reports of increased numbers of Substance P positive nerve fibres in the bladders of patients with interstitial cystitis, compared to controls (Pang et al 1995). VVS has been associated with interstitial cystitis as there is common embryological derivation for the bladder and the vulval vestibule, and indeed VVS has been proposed as a generalised disorder of urogenital sinus derived endothelium (Fitzpatrick et al 1993).

A study looking for the immunofluorescence of complement activation (previously seen in interstitial cystitis), in women with VVS, found a similar pattern and suggested that Substance P may trigger activation of the complement cascade via endothelial damage (Stewart and Berger 1997).

It is feasible that these women are unable to mount an adequate immune response, that then has a modulatory role on the neural pathways of chronic pain. Study of patients who do not require surgery to gain improvement in symptoms, but respond to conservative management, may clarify whether cytokine expression returned.

## **Conclusion**

The decreased expression of all 3 cytokines in women with VVS compared to normal controls was unexpected, as VVS has been postulated as an inflammatory condition. However, these findings concur with the decreased expression found in the areas of highest algia in women with VVS as previously described by Foster and Hasday (1997).

This argument is also supported by previous evidence that VVS is not inflammatory (Bohm-Starke 2001, Slone 1999).

It is not clear whether the apparent lack of TNF $\alpha$  and IL-1 expression in this study is a result of the pathogenesis of VVS or a predisposing factor, but as a result of this current study, VVS may no longer be regarded as the result of a local inflammatory process.

If VVS is not an inflammatory condition, then it is necessary to investigate possible causes from a new perspective.

## **CHAPTER FIVE - IS VULVAL VESTIBULITIS SYNDROME A HORMONAL CONDITION?**

### **Introduction**

The possibility of a relationship between VVS and hormonal factors has been questioned, although not studied extensively. Two studies have investigated whether oral contraceptive pill (OCP) use affects the risk of developing VVS.

In Canada, Bazin et al (1994) performed an exploratory case control study to evaluate the association of potential risk factors with occurrence of VVS. Fifty-seven women fulfilling Friedrich's criteria were recruited and compared to 173 controls without dyspareunia. OCP use showed some association with relative risk (RR) of developing VVS. Compared to women who had never used the OCP, former and current users had RR of developing VVS of 4.6 and 6.8 respectively. Early use of the OCP increased the risk further, with women who had used the OCP before the age of 17 having a RR of 11.0 (95% confidence intervals (CI) 1.3 – 97.1) compared to women who had never used the OCP. Duration of use did not appear to affect relative risks. Early menarche was also associated with increased risk (RR= 1.8 CI 0.8 – 4.0).

The authors suggested that factors occurring early in reproductive life are relevant and these may well be hormonal. A possible explanation was offered: the oral contraceptive pill has an effect on glandular components and vestibular mucus secretion, which play a role in coital lubrication. They therefore proposed that mucus protects the vestibular epithelium against physiological secretions and that alterations in this protection lead to a chronic irritant effect, which leads to dyspareunia.

A further epidemiological case control study also found an association with OCP use (Sjoberg et al 1997). Thirty-two women were compared to 17 healthy controls, and it was found that cases of VVS had used the OCP for a significantly longer period of time.

The authors suggested that the hypothesis proposed by Bazin et al was contradictory, considering most patients improve with vestibulectomy, which removes the glands. They suggested that if these glands were important then it would be expected that the condition would worsen if the glands were removed.

The same authors had previously demonstrated down regulation of oestrogen receptors (ER) by the OCP (Sjoberg et al 1989). They proposed that prolonged use, or use early in life, leads to increased down regulation of ER and lower levels of ER lead to the vestibular epithelium becoming thin and fragile and therefore irritated by the acidic vaginal pH.

Two authors have described the use of topical oestrogen cream as treatment for VVS with some success (Bazin et al 1995, Willems 1999).

Bazin et al performed a randomised double-blind placebo controlled study using vaginal oestrogen cream. Sixty-six percent of patients improved compared to 48% in the placebo arm. This was not a statistically significant result; however, a statistically significant decrease in the erythema of the gland openings was noticed.

The study by Willems used a combination of oestrogen cream, biofeedback and low oxalate diet and so it is not possible to ascertain which treatment was the effective

component. Unfortunately, neither author proposed a mechanism linking VVS and oestrogen.

In the specialist Vulval Clinic at the Royal Free Hospital it was noticed anecdotally that many of the patients with VVS had hormonal factors that appeared to affect their symptoms. A proportion of women had been diagnosed with polycystic ovaries, following investigation for menstrual irregularities, with others reporting that pregnancy, or commencing or ceasing the combined OCP altered their symptoms. Women who had managed to become pregnant reported changes in the severity of their condition connected to breast-feeding, a known hypo-oestrogenised state.

In view of the apparent changes in symptoms with potential changes in oestrogen levels, we considered whether oestrogen could be implicated in the aetiology of VVS. The results presented later in this chapter, of serum oestradiol levels, led us to investigate oestrogen effects at the cellular level within the vulva.

Oestrogen can have an immunomodulatory effect, as it can stimulate antibody response but inhibit T cell mediated inflammation (Jossefson et al 1992). It has been shown that if markers of inflammation are measured before and after oophorectomy they rise. Equally, replacement of oestrogen post surgery corresponds to a reduction in levels of inflammatory markers (Pacifici et al 1991).

In women who are taking the OCP, endogenous oestradiol is suppressed (Gillmer et al 1980). Equally during the pill free interval serum oestradiol rises and by the seventh

day of the cycle some women have similar oestradiol concentrations to untreated women in the early follicular phase of a natural menstrual cycle (Cohen 1979). This would concur with a study performed at the Royal Free Hospital looking at follicular development in the pill free interval, as increasing oestradiol is the result of follicular development. One hundred and twenty women were scanned at the end of the pill free interval and 23% were found to have significant follicular development that could lead to ovulation (Tayob 1990).

On the basis of the above data, I postulated that women with VVS exhibit a degree of abnormality either of serum oestrogen levels or oestrogen receptor expression within the vestibule, effectively making them unresponsive to local circulating oestrogen, so that inflammation is not suppressed

### **Oestrogen receptors**

Discovered by Elwood Jensen in 1958 the oestrogen receptor (ER) is a ligand activated transcription factor that mediates the effects of the steroid hormone 17-beta-oestradiol in both males and females. It has the characteristic structure of a member of the nuclear hormone receptor superfamily. It has been shown that oestrogen receptors are present in both the vagina and vulva, using both biochemical (ELISA) and immunohistochemical methods (Garau et al 1986, MacLean et al 1990). Using a rat monoclonal antibody against human ER, in frozen tissue with a peroxidase-antiperoxidase technique, MacLean et al (1990) demonstrated ER expression in epidermal keratinocytes and dermal fibroblasts of the vulva and perineum. This



expression was at a much lower frequency and intensity of staining than that seen in the vagina. They were unable to detect any ER expression in the vulval stroma. As the concentration of oestrogen receptors decreases from the vagina out through the vestibular area to the vulval skin, it may be expected that the normal vestibule would express oestrogen receptors.

Similar results were later obtained by the same group, using a standard streptavidin-biotin technique on frozen sections, again using a rat monoclonal antibody against human ER (Hodgins et 1998). Semi quantitative analysis, using ER expression from 2 random fields, confirmed a significant decline in epidermal and fibroblast ER expression from the vagina out towards the vulva and perineum. The group did not find ER associated with blood vessels, implying that oestrogen does not have a role to play in the vasculature of the vulva. Distribution of oestrogen receptors in both the vulva and vagina did not differ between pre and postmenopausal women.

Progesterone receptors did not appear to be present in the vulva, although they were found in the vagina.

Initially it was thought there was only one oestrogen receptor but in recent years a second subtype of receptor has been discovered (ER $\beta$ ) and so the original oestrogen receptor is now known as ER $\alpha$  (Paech et al 1997). The work demonstrating the presence of ER in the vulva used an antibody that does not cross react with ER $\beta$ , and so it is assumed that ER in the vulva is of the ER $\alpha$  subtype.

In view of the fact that some of the patients with VVS seen in the Vulval Clinic appeared to respond to topical oestrogen, it was decided to conduct two studies: the

first to investigate ER expression in patients with VVS, compared to normal controls, and the second to see if there was any relationship between ER expression and response to topical oestrogen therapy.

## **Study 1 – Investigation of ER expression in VVS**

### **Materials and Method**

#### **Tissue and serum samples**

Serum oestradiol levels in a group of women with VVS (n=105) were measured and compared to levels in a group of normal controls (n=57). The normal controls were patients without any vulval symptoms who were having unrelated surgery. The mean age of the VVS patients was 34.6 (range 22 – 53) and the mean age of the control group was 32.3 (range 20 – 46). The serum samples were linked in both groups with the time of the subject's menstrual cycle. Of the 105 blood samples taken from women with Vulval Vestibulitis Syndrome, 73 were not using hormonal contraception, and 8 of these were postmenopausal.

57 control samples were taken from premenopausal women, who were not using hormonal contraception.

Serum oestradiol ELISA assays were performed on all samples and the results of the 2 groups compared.

Tissue was obtained from women with a diagnosis of VVS (n=30) who fulfilled Friedrich's triad. This was either from women whom were undergoing vestibulectomy or from punch biopsies taken in the vulval outpatient clinic.

The directed biopsies were taken under colposcopic guidance from the vulval vestibule over the area of one of the entrances to the lesser vestibular glands. In patients undergoing vestibulectomy, the whole of the vulval vestibule was removed (Marinoff and Turner 1991).

Following ethical approval, control tissue was obtained from patients (n=20) undergoing unrelated gynaecological surgery from whom permission had been granted to remove a 4mm punch biopsy from the vulval vestibule whilst under general anaesthetic. Outpatient biopsies were taken under local anaesthetic using 2ml of Citanest and Octapressin (Astra). Punch biopsies were obtained using a Stiefel punch with a diameter of 4mm.

All of these women were asymptomatic and not taking hormonal contraception. Six of the study group were currently using the OCP.

All of the control group and all but one of the study group were premenopausal. In both groups all the women were Caucasian and the controls were selected to age match the study group.

Haemostasis was obtained with either silver nitrate sticks or Monsel's solution.

The tissue was placed in neutral-buffered formalin and then paraffin wax-embedded.

Five micrometer sections were cut and one section was stained with Haematoxylin and Eosin (H&E) and the histopathology reviewed by a consultant gynaecological pathologist, to exclude other vulval pathology.

All sections were placed on 3-aminopropyltriethoxysilane-coated glass slides.

Positive control tissue for ER $\alpha$  staining was from a known ER $\alpha$  positive breast cancer.

Positive control tissue for progesterone receptor (PR) staining was from a known PR positive breast cancer. The antibodies used are summarised in Table 5.1

Table 5.1 ER $\alpha$  and progesterone antibodies

Receptor	Antibody	Dilution
ER $\alpha$	Clone ID5 Cat No. M7047, DAKO Cambridgeshire, UK	1:50
Progesterone	Clone 1A6 Cat No. NCL-PGR, Novocastra, Newcastle upon Tyne, UK	1:200

Antigen retrieval was performed by microwaving the sections for 20 minutes in 10mM sodium citrate buffer (pH 6.0). Primary antibody was then incubated for 1 hour at room temperature, followed by the biotin linked secondary. Negative controls had the primary antibody omitted. Stain was detected using streptavidin-biotin horseradish peroxidase complex (Dako, K0492) and 3,3'- diaminobenzidine. The sections were counterstained with Mayer's Haemalum (Merck, Lutterworth, Leics. UK) to define the cell nucleus and analysed under light microscopy.

Nuclei that were positive for ER $\alpha$  or PR stained brown and nuclei that were negative for ER $\alpha$  or PR stained blue.

The full immunohistochemical protocol is shown in Appendix 2.

### Scoring Methods

Two methods for quantifying the number of oestrogen receptors present were studied. The sections were analysed independently by 3 observers. Concurrence of opinion was defined as less than 5% variation in result.

If there was discrepancy of more than 5% in the results, the slides were reviewed by all observers and consensus of opinion obtained.

The first method was to take two random fields under a magnification of x200 and count the number of nuclei that stained positive in both the basal epidermis and the dermis, and express them as a percentage.

The intensity of the staining was also assessed and graded, with 1 being the weakest intensity and 4 being the strongest. These two numbers were then multiplied to give a final score for each field. The grading of intensity of staining is subjective, although previous studies have shown it to be reproducible (Hodgins et al 1998). Strict protocols related to concentration of solutions and timing of steps in the staining protocols were adhered to for each batch of slides processed.

In the second method, the whole sample was assessed. A field measuring scale was inserted into the eyepiece of the microscope and the total length of the epidermis was

measured. Then the length of epidermis with positive staining nuclei was measured. Therefore it was possible to calculate the number of positive staining epidermal nuclei in each section as a percentage of the total basal layer, to produce a staining index for each section. This was performed for both ER and PR. These indices were then put into groups (e.g. 0 – 10%, 10 – 20% etc.).

This second method was found to be more accurate, as it assessed the entire specimen, and therefore was used to calculate the results.

### Statistics

Statistical analysis was performed using a Minitab Statistics package for Windows. Data were analysed using Chi Squared or Mann Whitney U tests.

## Results

### Serum oestradiol

Six of the fifty-seven control samples (10.5%) had serum oestradiol levels lower than the reference ranges for that point in the menstrual cycle.

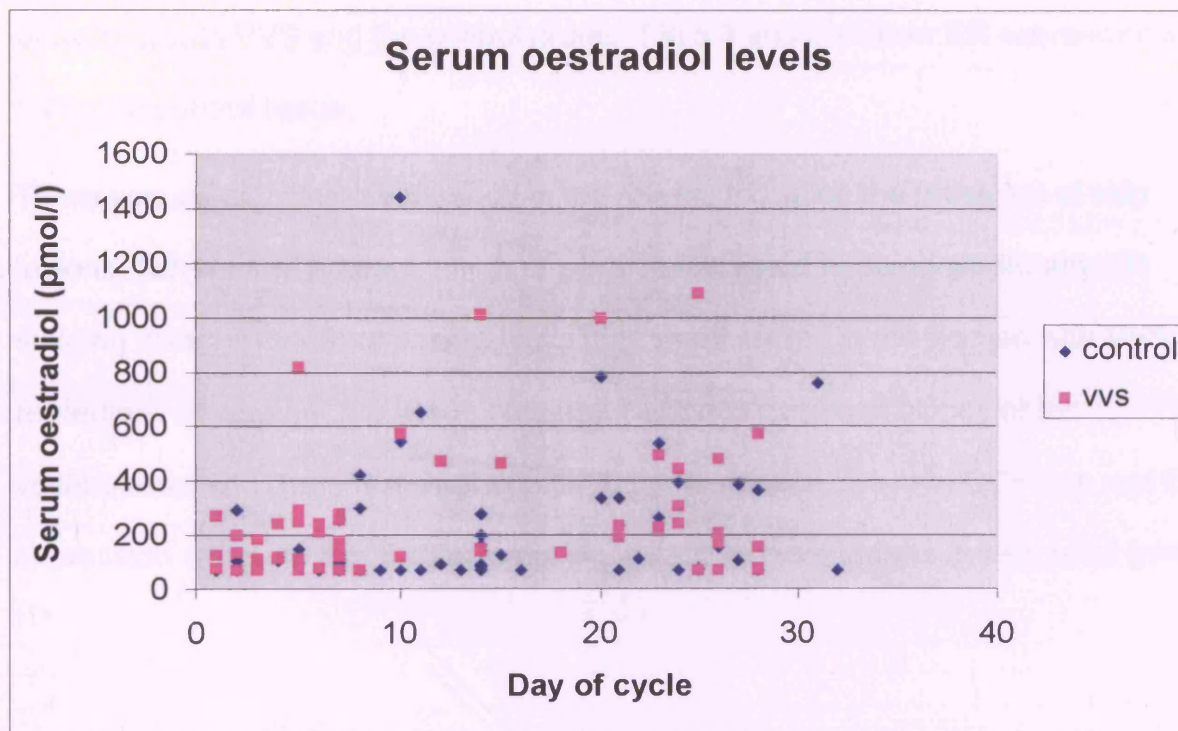
Twenty six (25%) of the patients with VVS had abnormally low oestradiol levels for that point in their menstrual cycles.

However, if the patients who were taking hormonal contraception, and therefore suppressing their endogenous levels of oestradiol, were excluded then only 9 samples

(8.6%) were outside the reference ranges. This is not statistically significant difference compared to the control group, using a Chi-Squared test.

The serum oestradiol results from women not taking hormonal contraception, are shown in Table 5.2 and represented graphically in Fig 5.1

Fig 5.1 Serum oestradiol of VVS and controls



### **ER $\alpha$ Expression**

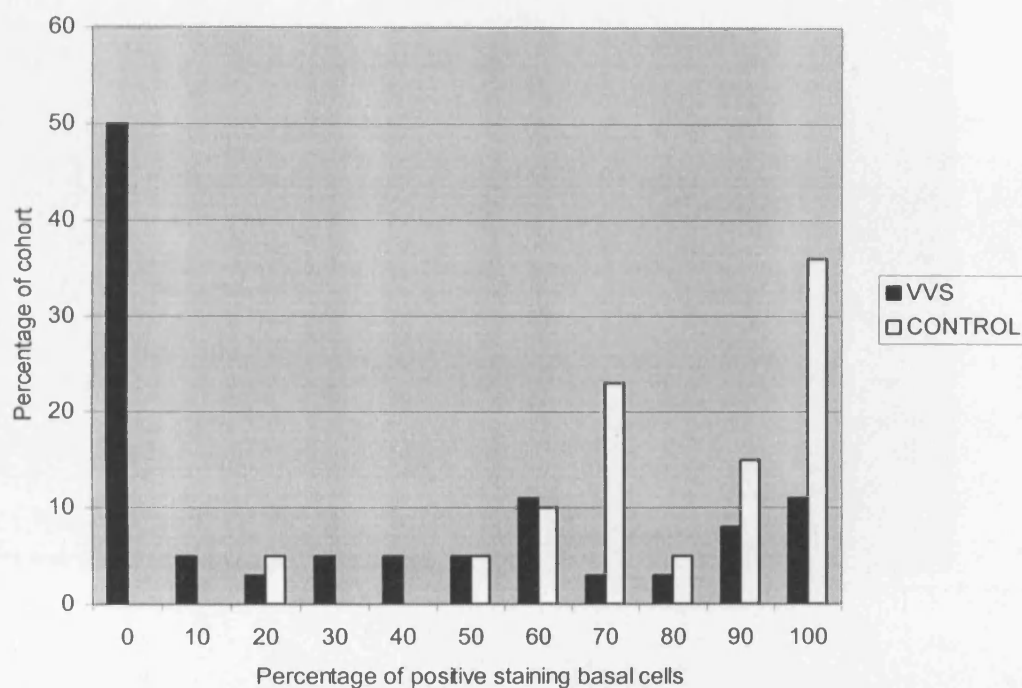
In the control group all the tissue samples exhibited ER $\alpha$  expression, with the majority showing more than 50% positive basal cells. In the group with VVS, 50% of the tissue samples did not exhibit any expression of ER $\alpha$ . Equally in the VVS tissue that did show ER $\alpha$  expression, this was confined to fewer basal cells, compared to the controls.

Fig.5.2 shows the differences in expression of oestrogen receptors between the group of women with VVS and the control group. Fig 5.3 and 5.4 show ER expression in VVS and control tissue.

There was no significant difference in the results, including the presence of skip lesions, (where a significant length of basal nuclei failed to demonstrate any ER staining within a length of positively staining basal nuclei) in the women with VVS, regardless of whether the tissue obtained had been by punch biopsy or by vestibulectomy. There does not appear to be correlation between OCP use and ER expression although this finding is not significant as the numbers are so small (n=6).



Fig 5.2 ER $\alpha$  expression in VVS and controls



Analysed using a Chi Squared test, the difference between cases and controls was statistically significant ( $p=0.0003$ ). The staining of the oestrogen receptors was predominantly in the basal layer of the epidermis, although some stromal staining was evident in some of the samples in both groups.

Fig 5.3 Vestibular tissue showing no ER expression

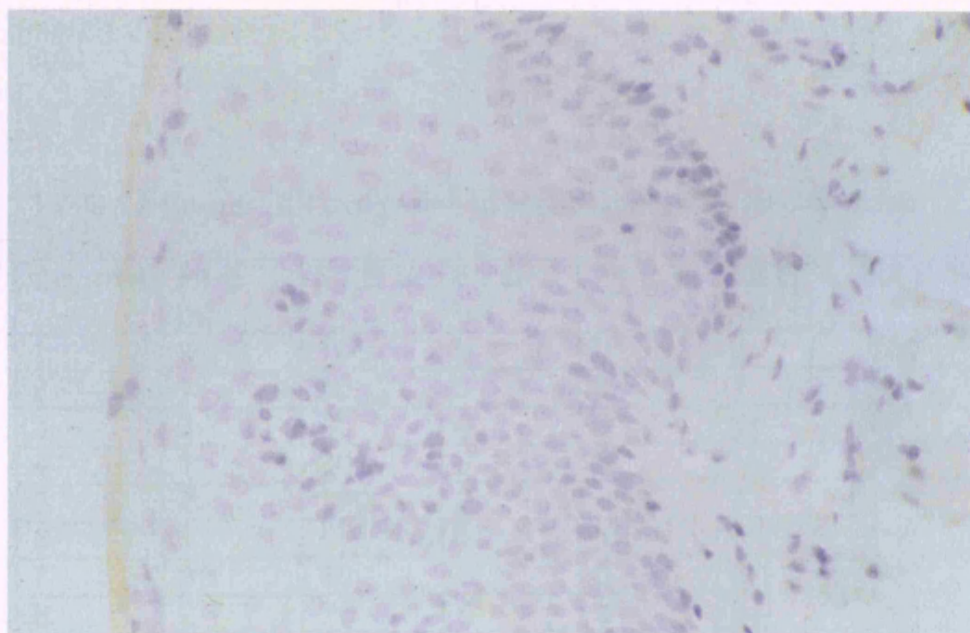


Fig 5.4 Vestibular tissue showing ER expression



The results obtained using the two different scoring methods are shown in Tables 5.2 - 5.4.

Table 5.2 Results: ER expression in control tissue in 2 random fields

Control	F1 E Int	F1 E %	F1 D Int	F1 D %	F2 E Int	F2 E %	F2 D Int	F2 D %
1	3	100	0	0	3	100	0	0
2	1	50	1	20	1	50	1	5
3	2	70	0	0	2	50	1	10
4	1	30	0	0	1	25	0	0
5	2	100	2	<5	3	100	0	0
6	1	5	1	10	4	100	0	0
7	3	40	0	0	3	100	1	10
8	2	75	0	0	3	85	2	<5
9	3	100	0	0	4	100	2	<5
10	2	100	1	5	4	100	2	5
11	2	80	1	<5	2	50	0	0
12	4	100	2	<5	3	75	0	0
13	1	40	1	50	2	75	0	0
14	4	80	1	5	4	10	2	10
15	4	100	3	10	4	100	2	20
16	4	100	0	0	3	100	0	0
17	4	100	2	15	4	100	0	0
18	3	100	0	0	2	80	0	0
19	1	100	0	0	1	100	0	0
20	3	100	0	0	3	100	1	<5
21	4	100	0	0	4	100	1	10

Key:

F1 = Random Field 1

E Int = Epidermal Intensity

D Int = Dermal Intensity

F2 = Random Field 2

E % = Epidermal percentage staining positive

D % = Dermal percentage staining positive

Table 5.3 Results: ER expression in VVS tissue in 2 random fields

<b>SAMPLE</b>	<b>F1 E Int</b>	<b>F1 E %</b>	<b>F1 D Int</b>	<b>F1 D %</b>	<b>F2 E Int</b>	<b>F2 E %</b>	<b>F2 D Int</b>	<b>F2 D %</b>
979632	4	75	4	20	4	50	3	25
9714579	2	60	0	0	1	50	1	30
95436	0	0	0	0	0	0	0	0
975839	3	100	2	70	3	95	2	80
921212	0	0	3	95	0	0	4	95
190697b	1	0	0	0	0	0	3	10
946844	1	10	0	0	0	0	0	0
956595	3	50	0	0	2	30	0	0
943708	3	10	2	5	1	20	2	30
961552	0	0	0	0	1	10	0	0
94419	4	90	1	0	3	60	0	0
968886	0	0	0	0	0	0	0	0
953949	2	85	0	0	1	80	0	0
95394	2	85	0	0	2	90	0	0
94861	3	80	0	0	0	0	0	0
943709	4	25	3	50	4	20	0	0
9610453	4	10	2	10	4	70	0	0
963371	0	0	0	0	0	0	0	0
975954	0	0	0	0	0	0	0	0
976017	2	20	1	10	1	30	0	0
978704	1	10	0	0	0	0	0	0
974024	0	0	0	0	0	0	0	0
190697	0	0	0	0	0	0	0	0
190697c	0	0	0	0	0	0	0	0
9711579	1	20	0	0	1	10	0	0
9713276	2	30	1	50	1	10	0	0
971668	0	0	0	0	0	0	1	50
94196	0	0	0	0	0	0	0	0
9712432	1	60	0	0	1	50	0	0
978868	0	0	0	0	0	0	0	0
97474	0	0	0	0	0	0	0	0
9712093	3	60	0	0	4	60	0	0
954773			0	0			0	0
975629	0	0	0	0	2	10	0	0
973858	0	0	0	0	0	0	0	0
916353	2	10	0	0	3	50	0	0
972740	4	80	0	0	4	50	0	0
975839	4	10	0	0	4	10	0	0

Table 5.4 Results: ER expression as percentage of total length of sample

SUBJECT	SAMPLE	RESULT (% +VE)	CONTROL	SAMPLE	RESULT (%+VE)
975954	P	0	1	P	90
976017	V	45	2	P	60
921212	V	0	3	P	70
94419	V	100	4	P	25
978704	P	10	5	P	100
971668	V	0	6	P	75
963371	V	0	7	P	78
974024	V	0	8	P	80
946844	V	0	9	P	100
94861	V	0	10	P	97
943709	V	92	11	P	75
961552	V	0	12	P	75
956595	V	37	13	P	67
972740	V	50	14	P	53
190697	P	0	15	P	100
190697b	P	0	16	P	100
190697c	P	55	17	P	100
979632	P	66	18	P	95
9711579	P	25	19	P	100
943708	V	0	20	P	100
954773	V	30	21	P	100
916353	V	42			
9712093	V	58			
975629	P	50			
9713276	P	100			
953949	V	10			
973858	P	0			
9712432	P	70			
94169	V	100			
978868	V	0			
97474	V	0			
9610453	P	100			
968886	P	0			
95436	V	0			
9714579	P	0			
975839	V	95			

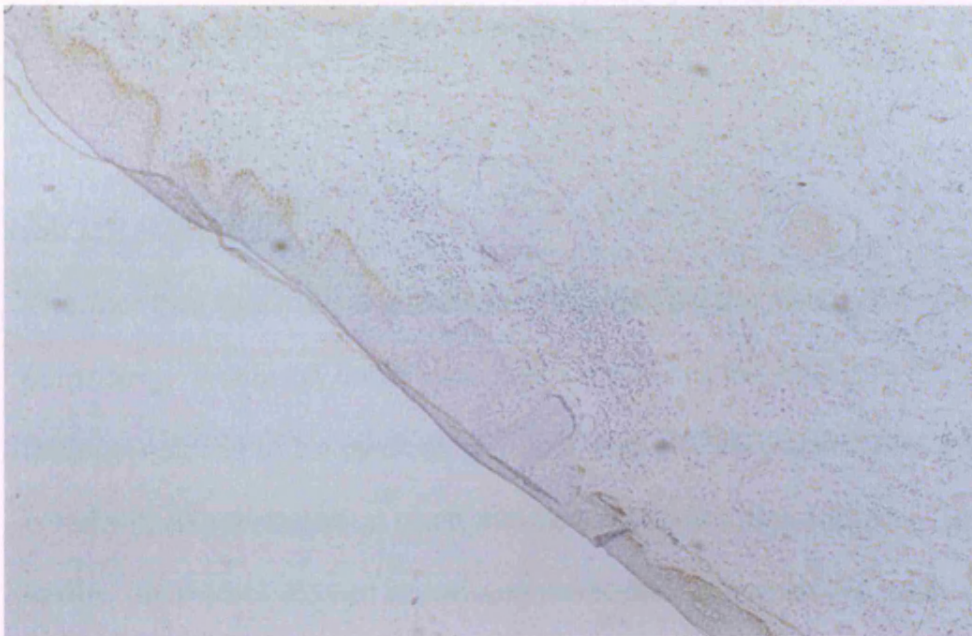
P = Punch biopsy

V = Vestibulectomy specimen



A phenomenon seen in the tissue samples of the group with Vulval Vestibulitis Syndrome but not in the control group, was the appearance of apparent “skip lesions” in the staining of the basal layer. ER\_ expression was found in patches, with negative staining areas in between. It was noted that often there was an area of lymphocytic infiltration below the negative areas, indicating an area of chronic inflammation. An example of a skip lesion is shown in Fig.5.5.

Fig 5.5 A “skip lesion”



### **PR Expression**

No PR expression was demonstrated in the vestibular samples from either the control group or from the women with Vulval Vestibulitis Syndrome.

## **Discussion**

There has been little work examining VVS at a cellular level. The majority of investigations have concentrated on the histopathology of this condition, and have remained inconclusive. Two recent studies have attempted to explore VVS at this level by analysing inflammatory markers associated with this condition (Foster et al 1997, Jeremias et al 2000), but serum oestradiol or tissue oestrogen receptor expression have never been investigated. Inflammatory markers and VVS are discussed in greater detail in Chapter 4.

## **Serum oestradiol**

The fact that there is no correlation between serum oestradiol and VVS is not surprising. Although there have been reports in the literature that some women find their symptoms to be cyclical, the definition of VVS implies that the symptoms are constant, exacerbated at each attempt at vaginal penetration. The women with low serum oestradiol did not report any menopausal symptoms in either the control or study groups. This is not surprising, as previously discussed, women taking the oral contraceptive pill have their endogenous oestradiol suppressed, and they do not suffer with menopausal symptoms. The results here reflect endogenous serum oestradiol levels, as there is no cross reactivity between ethinyloestradiol and oestradiol 17 $\beta$  (personal communication, Professor Back, Department of Pharmacology and Clinical Therapeutics, University of Liverpool).

Although mainly affecting premenopausal women we recognise that VVS is seen, although uncommonly, in older postmenopausal women and this group is represented in our cohort. Equally, in patients who report improvement in their condition when pregnant, we cannot conclude that increased serum oestrogen is responsible.

In view of the lack of systemic symptoms, it is more logical that any hormonal connection with VVS is more likely to be at a local level. We know that the signs of VVS are elicited in very specific sites with the rest of the vulva not involved.

### **Progesterone receptors**

The lack of PR expression in the vestibule concurs with previous work showing PR expression in the vagina but not in the vulva (Hodgins et al 1998).

Embryologically the vestibule and the vulva are derived from the same origin, the urogenital sinus. Therefore it is not surprising that vestibular tissue, as well as vulval tissue, does not express PR, whereas vaginal tissue, which is Mullerian in origin, does express PR.

### **Oestrogen receptors**

The results from this part of the study are possibly of greater relevance to this condition.

Two methods for quantifying the number of oestrogen receptors present were initially used. The first was the use of random fields and grading of number of receptors and intensity of nuclear staining. The second was to measure the whole length of the sample and to calculate the percentage of positive cells in the basal layer. The



disadvantage of the first method was that on some of the samples there appeared to be 'skip lesions' in which there was a sudden cut off between positive and negative nuclei. Thus, if two random fields were selected it was possible to obtain a negative result by chance, from a sample that was actually predominantly positive.

The second method was chosen as the preferred method of assessment in this study as it removed the possibility of choosing at random an area that was not representative of the whole sample. It allowed assessment of the entire section of the slide and allowed skip lesions to be recorded.

Given the marked difference in ER $\alpha$  expression between the 2 groups, and decreased expression in VVS patients, this initial study has raised a variety of questions to be investigated further. We do not know if the skip lesions can be related to areas of inflammation, thus supporting the theory that inflammation can be suppressed by the use of oestrogen, but in view of the previous chapter's findings, this may not be the case. This could perhaps predict the success of treatment.

Both topical steroid preparations and topical oestrogen are widely used as medical management of VVS (Bazin et al 1995, Willems 1999, Sonnex 1999). However if a patient does not have local oestrogen receptors, then topical oestrogen will be ineffective.

As to why some women respond to local topical oestrogen remains unclear. Certainly these women are not menopausal but it appears their endogenous oestrogen is

insufficient. I believe there is a local problem with the tissue and therefore circulating hormones are not relevant.

It has previously been shown that the OCP can downregulate ER in the vulva (Sjoberg 1989), although this was not seen in my study. We also know that the OCP produces a hypo-oestrogenised state, as previously discussed. Could it then be possible that high dose local oestrogen could upregulate the ER and improve patient's symptoms? This would be an interesting future study to investigate these effects.

Another possibility that has been proposed is that the tissues are fragile due to insufficient oestrogen and replacing the oestrogen improves the quality of the tissues (Bazin 1994), and this could be explained by the mechanism described above.

A third explanation is offered by Bohm-Starke et al (2004), who demonstrated that women on the OCP have a lower mechanical pain threshold and that oestrogen is a potent pain modulator, therefore it is possible that topical oestrogen somehow modulates pain perception and reduces the allodynia experienced. The same group had previously demonstrated increased nociceptor sensitisation (Bohm-Starke et al 2001) and it is possible that oestrogen could modulate this sensitisation.

Subsets of VVS have been proposed in the past (Goetsch 1991) but these have referred to onset of symptoms rather than different aetiological factors. If there were a subset of women with VVS who have abnormal oestrogen receptor expression, then this would explain why not all patients would respond to topical medical therapy and therefore require surgical intervention.

Previous medical treatment may down regulate ER expression and further controlled study is needed. In our population, medical treatment had been used for some of the patients previously, with some patients receiving multiple treatments before referral. The majority of the specimens obtained as outpatient punch biopsies had used topical steroids previously, but had been treatment free prior to biopsy. The vestibulectomy specimens were from women who had not responded to multiple medical treatments, including topical steroids and oestrogen, but all patients had ceased medical treatment several months prior to surgery. As none of the group was undergoing active treatment at the time the biopsies were taken, the lack of ER expression seen in this study probably cannot be explained by the theory of down regulation.

## **Conclusion**

An understanding of the oestrogen receptor expression in women with VVS may allow us to predict which patients are likely to respond to specific treatments, allowing the clinician to improve management.

Using this theory I then performed a further analysis to investigate response to treatment and whether it correlated with ER expression.

## **Study 2 - Treatment response and ER status**

### **Materials and methods**

The 30 women with VVS from whom the biopsies had been taken were followed during the study to assess ultimate response to treatment.

Response to treatment was assessed subjectively by patient opinion and measured objectively with the algometer.

Successful treatment was defined as the treatment to which the patient responded to the point they were able to have intercourse, which led to their discharge from the clinic.

The protocol for treatment of VVS at the Royal Free at this time is summarised below:

Examination and swabs were performed to exclude other conditions.

Initial treatment was Trimovate ® cream (clobetasone butyrate 0.05%, oxytetracycline 3%, Nystatin 100 000units/g) applied nightly for 8 weeks to the vestibule. In order for accurate treatment the vestibule was shown to the patient by use of a colposcope and a closed circuit television link to a monitor. This enabled the patient to identify the area that needed treatment.

At the first clinic visit the patient was counselled about her diagnosis. Difficulties in recognition and treatment was discussed, as often the women had seen multiple clinicians and offered multiple treatments. The treatment protocol was explained in

detail, emphasising that relatively few women eventually required surgery. General advice was given about underwear, toiletries and contraception, to minimise potential allergens during treatment.

Although there was not a psychosexual counsellor attached to the clinic, patients were advised this was available within the hospital if required and the opportunity for the patient's partner to attend the clinic was offered.

Written information was given to take home, about vulval vestibulitis syndrome and support groups, such as the Vulval Pain Society were discussed.

The patient was provided with the telephone number of the investigator, should they wish to contact the clinic.

Patients were reviewed after 2 – 3 months. If there had been little or no response to steroids, then a course of topical oestrogen cream (Premarin ®) applied nightly for 8 weeks was prescribed.

Vestibulectomy was performed only for women who had failed all medical treatment and constituted less than 10% of all new referrals of women with VVS.

Vestibulectomy was performed under a general anaesthetic. The vulval vestibule was identified and the margins marked with a carbon dioxide laser, extending anteriorly in a horseshoe shape to include both paraurethral glands, and posteriorly to the fourchette at the outer margin and the hymenal ring at the inner margin. The vestibule was then removed, either by sharp dissection, or using the laser to a depth of 3 –

5mm. The vagina was then undermined and mobilised to cover the defect. Haemostasis was achieved and the vagina sutured to the vulva using interrupted 2/0 undyed vicryl®. Local anaesthetic was infiltrated and the vulva covered with a Gelonet® dressing and a urethral catheter inserted overnight. Most patients were discharged on day 2.

Unfortunately some of the earlier vestibulectomy specimens were retrieved from archival tissue. These patients had not benefited from sequential treatment, or had been referred specifically for surgery from other units and this accounts for the larger than expected proportion of surgical samples compared to outpatient punch biopsies.

### Case note review

To confirm our experience that few women with VVS ultimately are treated by surgery, the treatment and outcome of all patients seen during the study period of 1997 – 1999 were reviewed. All case notes and research databases were reviewed to establish outcome data. Where data were not available, the patients were contacted to establish outcome.

### **Results**

Of the 30 women who had biopsies, 1 went elsewhere for treatment and so no follow up data is available. Out of the remaining 29 patients, 3 responded to Trimovate, 7 responded to Premarin and 19 responded to surgery. The ER status of each of these groups was analysed and shown in Table 5.6 and summarised in Table 5.7. Six of the

samples were from archived tissue and therefore had not been through the sequential treatment protocol, and so these were excluded from the analysis.

Table 5.5 ER status and treatment response

<b>SUBJECT</b>	<b>ER status</b>	<b>Treatment responded to</b>
975954	Negative	Premarin
973858	Negative	Trimovate
921212	Negative	Surgery
971668	Negative	Surgery
963371	Negative	Surgery
946844	Negative	Surgery
94861	Negative	Surgery
943708	Negative	Surgery
978868	Negative	Surgery
97474	Negative	Surgery
968886	Negative	Surgery
95436	Negative	Surgery
9714579	Positive	Premarin
976017	Positive	Premarin
978704	Positive	Premarin
9711579	Positive	Premarin
9713276	Positive	Premarin
9712432	Positive	Premarin
9610453	Positive	Trimovate
979632	Positive	Trimovate
975839	Positive	Surgery
943709	Positive	Surgery
956595	Positive	Surgery
94419	Positive	Surgery
954773	Positive	Surgery
9712093	Positive	Surgery
975629	Positive	Surgery
953949	Positive	Surgery
94169	Positive	Surgery

Table 5.6 Summary of ER status and response to treatment

<b>TREATMENT</b>	<b>PREMARIN (n=7)</b>	<b>TRIMOVATE (n=3)</b>	<b>SURGERY (n=13)</b>
<b>ER<math>\alpha</math> POSITIVE</b> (n=11)	6	2	3
<b>ER<math>\alpha</math> NEGATIVE</b> (n=12)	1	1	10

This shows that in the group that did not express ER $\alpha$ , 83% (n=10) responded to surgery and 17% (n=2) responded to medical treatment, one responding to topical steroid and one responding to topical oestrogen.

In the group that did express ER $\alpha$ , 27% (n=3) responded to surgery and 73% (n=8) responded to medical treatment, two responding to topical steroid and 6 responding to topical oestrogen.

This concurs with our experience that the minority of patients will require a vestibulectomy to treat their condition.



### Patients discharged and response to treatment

During this period 109 new patients were seen with a diagnosis of vulval vestibulitis syndrome. The mean number of visits to the clinic was 3. Seventy patients had been discharged from the clinic and 39 had not been discharged.

Sixty-two of the patients said they had responded to treatment. When asked about their symptoms at their last visit to the clinic, 58 said that intercourse was pain free and 4 said that intercourse was possible but associated with a minor degree of pain.

The treatment the patients responded to is shown in Table 5.8.

Table 5.7 Treatment Response

<b>Treatment</b>	<b>Number of patients</b>
Trimovate	31
Premarin	14
Surgery	13
Other	4

The treatments in the “other” category were tricyclic antidepressants (n=1), Chinese herbs, which on further investigation were found to contain oestrogen (n=1) and low oxalate diet (n=2).

### Surgery

From the 109 patients presented with vulval vestibulitis syndrome 15 were offered surgery, as they had not responded to medical treatment. Thirteen had improved following vestibulectomy, 1 declined surgery and one patient's symptoms worsened following surgery. This patient was a tertiary referral from another unit who had declined sequential medical treatment and only wanted surgery. She later responded to topical oestrogen therapy.

### Patients not discharged

Of the 39 patients not discharged from clinic, 22 were still receiving treatment, 15 had not attended their follow up appointment and 2 had not attended follow up twice.

The patients who had not attended their follow up appointments who were contactable by telephone were asked why they had not attended follow up appointments. Their reasons are summarised in Table 5.9. I was unable to contact the 2 patients who had not attended twice.

Table 5.8 Reasons for not attending follow up

Reason not attended follow up	Number of patients
Improved with treatment given	5
Left country	3
Declined research participation	1
Declined surgery	1
Attended other vulval clinic	3
Requested endocrine referral	1
Pregnant	1

### **Assessment of response to Premarin**

Response to treatment was assessed in two ways, as previously described in Chapter

2. Patient's subjective perception of whether their symptoms had improved or worsened with treatment was recorded along with their ability to have sexual intercourse. Objective assessment of vestibular tenderness was assessed using the algometer, which we have previously shown correlates well with patient symptomatology.

Comparisons of the algometer readings before and after treatment with Premarin were made. The overall median algometer scores for the treatment group were calculated and compared to the scores recorded at the 1<sup>st</sup> visit to the clinic, prior to treatment.

Statistical analysis was performed using a Minitab Statistics package for Windows. Non parametric data were analysed with the Mann- Whitney U test.

Table 5.9 Comparison of algometer scores:1<sup>st</sup> visit and after Premarin

Comparison	Mean scores	P value
1 <sup>st</sup> visit / Premarin (n=14)	5.0 – 18.0	0.00008

This result is highly significant and shows there was a genuine improvement in vestibular tenderness in the groups using Premarin.

If subdivided further to look at the group that was discharged following successful responses to treatment, again the results are significant: these results are shown in Table 5.10.

Table 5.10 Premarin responders: Comparison of algometer scores before and after treatment

Comparison	Mean scores	P value
1 <sup>st</sup> visit / Trimovate	12 – 9	0.88
1 <sup>st</sup> visit / Premarin	7 – 15	0.005
Trimovate/Premarin	6 – 19	0.003

(N=14)

This shows there was genuine significant response to treatment with Premarin and that the patients who did respond to Premarin did not respond to Trimovate.

## **Discussion**

These results are encouraging, as it appears that there if the ER status of a patient is known, then it may help in predicting what treatment they may respond to.

Although topical oestrogen has been reported as being successful in some patients with VVS (Sonnex 1999, Willems 1999) until now there has not been any logical basis for its use.

It is also encouraging that most women will respond to treatment, although finding the correct approach can take time and this is particularly important in this group of women, who often have been searching for a cure for a considerable period of time. These data also highlight the need for logical sequential medical treatment, prior to

surgical intervention, which may not be necessary. This should be reiterated to patients, who may have attended a tertiary referral unit purely for a surgical opinion and may not be keen to participate in any further medical treatment, as the multiple treatments they have already tried elsewhere have been unsuccessful.

It is easy as a clinician to assume that if a patient does not return to the clinic it is because she is cured, and the follow up data in this study represent a more accurate picture of the reasons for non attendance, although in this case one third of the patients lost to follow up had actually improved, and therefore had not thought it necessary to return.

## **Conclusion**

Treatment response to topical oestrogen is likely if the patient does express ER $\alpha$ , and these patients are unlikely to respond to topical steroids. This may make decision making when tailoring treatment to a patients needs easier, but the need for larger prospective studies and follow up is recognised.

## **CHAPTER SIX - DISCUSSION**

VVS remains a frustrating and complex problem, both for the clinician trying to treat these women and the women who suffer with it. The lack of a definite pathological diagnosis, or a logical treatment algorithm means that frequently patient and clinician are left with uncertainty.

This thesis has attempted to clarify some of the previously uninvestigated areas of this complex condition, and although some questions have been answered, many more have been raised.

The objectives of this thesis were:

1. To review the current knowledge in the literature about VVS.
2. To define the epidemiological characteristics of our cohort of women and compare these to existing data.
3. To objectively quantify the pain experienced by women with VVS.
4. To explore whether VVS is an inflammatory condition.
5. To explore whether VVS has a hormonal component.

The data from my cohort are consistent with many characteristics common to other cohorts worldwide.

It is interesting that most women can identify a precipitating factor, and more work is needed to see what acts as a trigger to cause severe problems in a previously healthy

woman. It seems unlikely that vulvovaginal candidiasis is the cause, although over a quarter of the women believed such an episode precipitated their symptoms.

Although my cohort was not compared to a control group, it was not the intention of this study to compare the epidemiological differences between women with VVS and the normal population. This has been done in other studies and the concern of this thesis was whether this cohort compared to those elsewhere in the world, and whether environmental or cultural factors had any role. This does not appear to be the case, as the data contained in this thesis regarding epidemiological characteristics are comparable with groups from the US (Goetsch 1991) and Sweden (Sjoberg et al 1997). Again the most striking feature is that this condition does not appear to affect women who are not Caucasian. Although it may be expected that a cohort from Sweden would be mainly white, this is not the case in London. The question of genetic predisposition is certainly raised, and warrants further investigation.

This study has successfully quantified the tenderness experienced by women with VVS.

The algometer has proved to be a valuable asset, both in assessment of the condition, and monitoring progress with treatment. It is non-invasive, easy to use, and provides a definitive value for the tenderness experienced. As previously discussed in Chapter 2, Q-tip tenderness is very subjective, and user dependent. The algometer



has provided a reproducible method of assessment, which removes bias, both between clinicians and between different examinations.

The patients find it very acceptable, and its use enabled them to see improvement in a very real way, providing an excellent method of biofeedback.

It also proved to be a robust research tool, and enabled comparison of treatment modalities, which would not have been possible with subjective assessment.

The part of this thesis investigating whether VVS is an inflammatory condition has produced significant results, and added to the growing evidence that VVS is not the result of chronic inflammation and hence should not be treated as such. The implication, as a result of this evidence, is that VVS is not an “itis” and the renaming of the condition by the ISSVD in 2003 to localized provoked vulvodynia, may be more appropriate.

We do not know whether the decreased expression of the cytokines found in the vestibular tissue of women with VVS is the cause of the condition, or the result of the pathological process. It is unlikely to be causative, as the physical appearance of the vestibular glands is that usually seen with inflammation. If, however we are to assume that the erythema seen is not a result of an inflammatory process, but the result of increased blood flow to the area, due to neoangiogenesis, this could be explained.

Laser Doppler Perfusion Imaging (LDPI) has previously been used by a group at the Royal Free Hospital (Saravanamuthu et al) to investigate blood flow in the vulva. At

the start of this project, I did attempt to assess vestibular blood flow, using this technique, but no results were obtained due to the technical difficulties in accessing the vestibular area, without having to stretch adjacent tissues. The previous LDPI studies had concentrated on the labia majora and minora, which obviously are easier to access.

Future studies of angiogenesis including assessment of vascular endothelial growth factor (VEGF) would be useful, as it would be expected that these would be overexpressed in the vestibular area of women with VVS.

It has been shown there is increased innervation in the vestibule of symptomatic women (Bohm-Starke et al 1998, Westrom et al 1998), and it may be there is increased blood vessel formation associated with this neural proliferation.

The concept of increased innervation is interesting, as it gives an explanation for the hyperalgesia found in VVS. It also would explain why vestibulectomy appears to be successful, as the areas of neuronal proliferation are removed. This would provide the explanation for the need for surgery in some of these women, which previously had not seemed logical as to why removing apparently normal tissue helped to alleviate symptoms.

It is of course feasible that low levels of cytokines are the result of an inability to mount an immune response, but again this would have to be a local phenomenon, as these women are not systemically immunosuppressed.

It may be, however, that these findings are the result of an immunomodulatory process. We know that factors produced by neurones can potentiate expression of

nerve growth factor and downregulate expression of inflammatory associated cytokines, TNF $\alpha$  and IL-1 $\beta$  (Zhang and Fedoroff 1998). If there is neural proliferation, one assumes there will be increased neural growth factor, which can suppress cytokine production. This would complete the loop of this explanation, and again is an area for future exploration.

The explanation for women with VVS being unable to locally produce a normal reaction in the vestibule could again be applied to the lack of ER expression seen in this study. Again this result was highly significant, but this work is the first investigation of this kind in VVS, and so there are no other comparative results in the literature.

One of my initial hypotheses, that if oestrogen is an immunomodulator, suppressing inflammation, and the lack of ER means that oestrogen is not effective locally, is disproved if we now assume VVS is not an inflammatory condition. Moreover, my attempts to correlate ER expression with cytokine expression in the samples of women with VVS overall did not find any relationship.

As part of a future project, immunohistochemistry using double staining techniques, would allow direct comparisons of individual sections, to see if lengths of ER expression and cytokine expression were in the same or unrelated areas.

Another area of future investigation would be analysis in greater detail of the “skip lesions”. This phenomenon was also seen to some extent in the cytokine expression, where areas changed from positive to negative along the same length of epidermis. It would also be of value to see whether the areas of lymphocytic infiltration seen in the H&E sections, bore any relationship to the position of cytokine expression.

In view of the positive correlation between ER expression and the ability of the subject to respond to topical oestrogen, it may be worth considering whether vestibular biopsy and immunohistochemistry become part of the routine assessment of women with VVS.

The scientific investigation and published literature relating to VVS is expanding.

Studies have shown that this is not primarily a psychological condition, but as with any chronic pain syndrome these factors are obviously important. The question is whether these psychological features are a predisposition, causative, or a result of the disease. Whatever the psychological input, there is no doubt that VVS is a physical syndrome.

Early studies focussed on the pathology and searched for a single aetiological factor. The fact that so many studies are conflicting, and no single aetiological factor has been found, implies that instead of being a single condition, VVS may be the result of a heterogeneous series of events.

If this is the case, then this would explain why treatments have varied success.

Virtually every conceivable treatment modality has been used in the management of VVS, and even using similar protocols, results appear to vary between investigating groups.

Although surgery appears to be mainly successful, with most groups quoting success rates around 80 – 90%, it is the most invasive option and therefore not to be used as first line therapy. The need for a successful non invasive medical therapy is highlighted and should be the ultimate aim for clinicians involved in research in this area.

Achieving this aim, however, may not be possible until we understand the mechanism behind the disease process. Too many treatments are randomly applied, without a logical reason for doing so. For example, there is no longer convincing evidence regarding HPV involvement in the pathogenesis of VVS, and therefore to inflict painful interferon injections on women is not appropriate.

A clear distinction should be made between providing symptomatic relief and trying to cure the underlying problem. However, it should be recognised that for many of these women, who have been suffering for a number of years, a diagnosis and alleviation of symptoms, albeit temporary, may come as welcome respite.

More recently, the parameters of investigation have been widened. The role of molecular research techniques is beginning to emerge and this may hold the key to solving the problem of VVS.

The work looking at inflammatory markers by Foster and Hasday (1997) was the first of its kind to be published, at the same time as the initial work in this thesis was being performed. Work looking at other inflammatory markers (Bohm-Starke et al 2001, Slone et al 1999) has contributed to challenging the initial assumptions about the condition as an inflammatory process.

Genetic work (Jeremias et al 2000) is still in the preliminary stages, but no doubt will be expanded upon.

Recent work considering the neuroanatomy of the vestibule (Bohm-Starke et al 1998,1999, Warner et al 1996, Westrom et al 1998) is of interest, and the theories produced as a result of these studies again provide evidence that further molecular work is required.

The problem with most of these trials is that they are performed by individual units, with small numbers of patients, and are uncontrolled. It is therefore difficult to achieve statistically significant results. The need for greater collaboration and multicentred, controlled trials is paramount. Unfortunately, vulval disease has never been a high profile area of gynaecology, compared to oncology, infertility and incontinence.

The establishment of associations such as the BSSVD,ECSVD and ISSVD (British,European and International Societies for the Study of Vulvovaginal Disease) has helped to start encouraging national and international sharing of ideas and patient groups, and hopefully will lead to increased collaboration towards a common goal.

Certainly it appears there will not be a single explanation for VVS. I believe this is a heterogeneous syndrome, borne out by the fact that there appear to be subsets of women who respond to different treatments. It is certainly possible that women who respond to topical oestrogen have a different pathology to women who respond to topical corticosteroids, plus tetracycline. Although I feel that the theory of abnormal neuroanatomy could explain the group of patients who respond to tricyclic antidepressants, or surgery, perhaps a different explanation, or explanations, is needed for those who respond to medical treatment.

In conclusion, the studies within this thesis have succeeded in assessing characteristics of our study population and proving there is an objective form of assessment available for women with VVS. They have succeeded in showing that VVS is not an inflammatory condition, and there may be a hormonal component involved.

However, this work has also succeeded in creating more questions and suggesting further avenues for research to be explored in this complex condition.

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## **Appendix 1 Revised Classification of Vulvar Disease (ISSVD)**

### **1. INFECTIONS**

- Parasitic      e.g. Pediculosis, scabies
- Protozoal     e.g. amoebiasis
- Viral          e.g. herpes virus infection, condyloma acuminata
- Bacterial
- Fungal        e.g. candidosis, dermatophytosis
- Others

### **2. INFLAMMATORY SKIN DISEASE**

#### **Spongiotic disorders**

- Contact dermatitis
  - Irritant
  - Allergic
- Atopic dermatitis (acute and chronic)
- Seborrheic dermatitis
- Others

#### **Psoriasiform disorders**

- Lichenification (Lichen simplex)
- Atopic dermatitis (chronic)
- Seborrhoeic dermatitis
- Others

#### **Lichenoid disorders**

- Lichen sclerosus
- Lichen planus
- Fixed drug eruption
- Plasma cell vulvitis
- Lichenoid reaction, not otherwise specified (focal or diffuse)
- Lupus erythematosus
- Others

#### **Vesicobullous disorders**

- Pemphigoid
- Pemphigus
- Erythema multiforme
- Stevens Johnson Syndrome
- Others

#### **Granulomatous disorders**

##### **Non-infectious:**

- Sarcoidosis
- Crohn`s disease  
(Hidradenitis suppurativa)
- Others

##### **Infectious:**

- Tuberculosis
- Granuloma inguinale

## Vasculitis or related inflammatory disorders

- Leukocytoclastic

- Urticaria

- Aphthous ulcer

- Lymphoedema

- Behcet's disease

- Pyoderma gangrenosa

- (Fixed drug eruption)

- (Erythema multiforme)

- (Stevens Johnson Syndrome)

### **3.SKIN APPENDAGES DISORDERS**

- Hidradenitis suppurativa

- Fox - Fordyce disease

- Disorders of sweating

### **4.HORMONAL DISORDERS**

- Oestrogen

  - Excess - Precocious puberty

  - Others

  - Deficiency

    - Physiological

    - Lactation

    - Post menopausal

    - Others

    - Iatrogenic

- Androgen

  - Excess - Physiological

  - Iatrogenic

### **5. ULCERS AND EROSIONS**

- (Diseases that ulcerate and/or erode are listed according to histologic findings)

- Trauma

  - Obstetric

  - Surgical

  - Sexual

  - Accidental

  - Others (include fissures of the fossa navicularis)

### **6.DISORDERS OF PIGMENTATION**

- Hyperpigmentation

  - Melanin

    - Lentigo

    - Melanos vulvae (post inflammatory pigmentation)

  - Haemosiderin (post inflammatory pigmentation)

  - Vitiligo

- Hypopigmentation

  - Vitiligo

  - Post inflammatory pigmentation

## **Appendix 2 - Immunohistochemistry protocols**

### **Oestrogen Receptors**

- 1 Deparaffinise in xylene for 5 mins x3
- 2 Rehydrate in methanol for 5 mins x 3
- 3 Wash in distilled water (DDW) for 3 mins x 3
- 4 Wash in Phosphate Buffered Saline (PBS) for 3 mins x 2
- 5 Block endogenous peroxidase in 10% H<sub>2</sub>O<sub>2</sub>:  
(30ml hydrogen peroxide in 270ml DDW)  
Leave for 8 – 10 mins
- 6 Wash in PBS for 3 mins x 2
- 7 Microwave in citric buffer on full power for 20 mins
- 8 Allow citric buffer to cool for 30 mins
- 9 Wash in DDW for 3 mins
- 10 Block non specific binding with normal rabbit serum (NRS)  
Allow 250µl per slide ∴ dilution = 250 x number of slides  
NRS= Total ÷10 ∴ PBS-SAB = total – NRS
- 11 Pipette on to slides in 125µl quotients  
Leave for 15 mins
- 12 Add primary antibody (Monoclonal mouse anti-human ER Dako M7047,  
Clone 1D5) in dilution 1:50  
PBS-SAB only added to negative control  
Incubate for 1 hour in humidity chamber at room temperature
- 13 Wash in PBS for 3 mins x 3
- 14 Add secondary antibody (Rabbit anti-mouse biotin,) at dilution 1:200, diluted  
in PBS with 10% normal human serum  
Leave for 30 mins
- 15 Wash in PBS for 3 mins x 3
- 16 Add Streptavidin biotin complex (StreptABC/HRP Dako Duet kit K0492)  
diluted 1:200 in TBS  
Leave for 30 mins
- 17 Wash in PBS for 3 mins x 3
- 18 Incubate DAB (180mg DAB, 1000µl imidazole, 30 ml TBS, 260 ml DDW,  
120µl hydrogen peroxide)  
Leave for 8 – 12 mins
- 19 Wash in tap water
- 20 Counterstain with Mayers haemalum
- 21 Wash in DDW until clean
- 22 Wash in acid alcohol
- 23 Place in blueing solution
- 24 Dry
- 25 Glue coverslips onto slides

## **Tumour Necrosis Factor Alpha**

- 1 Deparaffinise in xylene for 5 mins x3
- 2 Rehydrate in methanol for 5 mins x 3
- 3 Wash in distilled water (DDW) for 5 mins x 2
- 4 Block endogenous peroxidase in 3% H<sub>2</sub>O<sub>2</sub>:  
Leave for 20 mins
- 5 Wash in TBS for 5 mins x 2
- 6 Block non specific binding with normal rabbit serum (NRS)  
Allow 250µl per slide
- 7 Pipette on to slides in 125µl quotients  
Leave for 10 mins
- 8 Add primary antibody (monoclonal TNF $\alpha$  – Santa Cruz Sc-7317, Clone 1E8-G6) in dilution 1:5  
PBS-SAB only added to negative control  
Incubate overnight at room temperature in humidity chamber
- 9 Wash in TBS for 5 mins x 2
- 10 Add secondary antibody (Rabbit anti-mouse Dako E0354) 1:200, diluted in TBS  
Incubate for 45 mins at room temperature in humidity chamber
- 11 Wash in TBS for 5 mins x 2
- 12 Add Streptavidin biotin complex (StreptABC/HRP Dako Duet kit K0492) diluted 1:200 in TBS  
Leave for 45 mins
- 13 Wash in PBS for 5 mins x 2
- 14 Incubate DAB (180mg DAB, 1000µl imidazole, 30 ml TBS, 260 ml DDW, 120µl hydrogen peroxide)  
Leave for 10 mins
- 15 Wash in DDW for 5 – 10 mins
- 16 Incubate DAB (180mg DAB, 1000µl imidazole, 30 ml TBS, 260 ml DDW, 120µl hydrogen peroxide)  
Leave for 8 – 12 mins
- 17 Wash in DDW
- 18 Counterstain with Mayers haemalum
- 19 Wash in DDW until clean
- 20 Wash in acid alcohol
- 21 Place in blueing solution
- 22 Wash in tap water
- 23 Dehydrate in methanol for 5 mins x 2
- 24 Clear in xylene
- 25 Mount in DPX mountant



## **Interleukin 1 beta**

- 1 Deparaffinise in xylene for 5 mins x3
- 2 Rehydrate in methanol for 5 mins x 3
- 3 Wash in distilled water (DDW) for 5 mins x 2
- 4 Block endogenous peroxidase in 3% H<sub>2</sub>O<sub>2</sub>, diluted 1:10  
Leave for 10 mins
- 5 Wash in TBS for 5 mins x 2
- 6 Block non specific binding with normal goat serum (DAKO X0907), diluted in TBS Allow 250µl per slide
- 7 Pipette on to slides in 125µl quotients  
Leave for 20 mins
- 8 Add primary antibody (Rabbit polyclonal – Santa Cruz Sc-7884) in dilution 1:100 PBS-SAB only added to negative control  
Incubate overnight at room temperature in humidity chamber
- 9 Wash in TBS for 5 mins x 2
- 10 Add secondary antibody (Goat anti-rabbit Dako K1492) 1:200, diluted in TBS Incubate for 45 mins at room temperature in humidity chamber
- 11 Wash in TBS for 5 mins x 2
- 12 Add Streptavidin biotin complex (StreptABC/HRP Dako Duet kit K0492) diluted 1:200 in TBS  
Leave for 45 mins
- 13 Wash in tap water
- 14 Counterstain with Mayers haemalum
- 15 Wash in DDW until clean
- 16 Wash in acid alcohol
- 17 Place in blueing solution
- 18 Wash in tap water
- 19 Dehydrate in methanol for 5 mins x 2
- 20 Mount in DPX mountant

## **Interleukin 1 alpha**

- 1 Deparaffinise in xylene for 5 mins x3
- 2 Rehydrate in methanol for 5 mins x 3
- 3 Wash in distilled water (DDW) for 7 mins x 2
- 4 Block endogenous peroxidase in 10% H<sub>2</sub>O<sub>2</sub>:  
(30ml hydrogen peroxide in 270ml DDW)  
Leave for 10 mins
- 5 Wash in PBS for 3 mins x 2
- 6 Block non specific binding with normal rabbit serum (NRS)  
Allow 250µl per slide ∴ dilution = 250 x number of slides  
NRS= Total ÷10 ∴ PBS-SAB = total – NRS
- 7 Pipette on to slides in 125µl quotients  
Leave for 10 mins
- 8 Add primary antibody (Monoclonal IL-1α, Santa Cruz Sc-9983, Clone B7) in  
dilution 1:20  
PBS-SAB only added to negative control  
Incubate overnight at room temperature
- 9 Wash in PBS for 3 mins x 3
- 10 Add secondary antibody (Rabbit anti-mouse biotin IgM Dako E0354) in  
dilution 1:200, diluted in PBS with 10% normal human serum  
Leave for 45 mins at room temperature
- 11 Wash in PBS for 3 mins x 2
- 12 Add Streptavidin biotin complex (StreptABC/HRP Dako Duet kit K0492)  
diluted 1:200 in TBS  
Leave for 30 mins
- 13 Wash in tap water
- 14 Counterstain with Mayers haemalum
- 15 Wash in DDW until clean
- 16 Wash in acid alcohol
- 17 Place in blueing solution
- 18 Wash in tap water
- 19 Dehydrate in methanol for 5 mins x 2
- 20 Mount in DPX mountant

## **Immunohistochemistry: Reagents and Buffers**

### **Xylene**

Merck, 'Analar' 102936H

### **Methanol**

Merck 'Analar' 101586B

### **Hydrogen Peroxide Solution**

Add 10 ml 30% hydrogen peroxide (Merck 'Analar' 101284N) to 90ml distilled water.

Final concentration = 3%

### **Phosphate buffered saline (PBS)**

Dissolve 5 PBS sachets (ICN 17-604-20) in 5l distilled water. Check pH.

### **Sodium Citrate Buffer**

Weigh out 2.1g citric acid monohydrate (Merck 'Analar' 100813M) add 950ml distilled water and 13ml 2M NaOH (Merck 'Analar' 1913833x). Make up to 1l. Adjust pH 6.0.

Store at 4°C.

### **PBS-SAB**

Weigh out 100mg bovine serum albumin (Fraction V, Sigma A-2153) and dissolve in 100ml PBS.

0.1%. Store at 4°C.

### **Normal Rabbit Serum (NRS)**

Dako X0902

### **Normal Goat Serum (NGS)**

Dako X9070

### **Normal Human Serum (NHS)**

Withdraw 9ml blood into serum tube. Allow to clot at 1 hour at room temperature.

Centrifuge (Centaur 2300rpm, 1800g) for 15 min. Remove supernatant. Heat at 56°C for 30 min (water bath) to denature complement. Store at -20°C in 500µl aliquots in Eppendorfer tubes

### **Tris-Buffered Saline (TBS)**

Weigh out 43.83g NaCl (Merck GPR 301235Q) and 30.6g Tris base (Merck 'Aristar' 452054C). Make up to 5l with distilled water. Add 35ml of concentrated HCl (Merck 'Analar' 101250D) to ensure pH is at 7.6 (in fume cupboard). Store at 4°C.

0.05M Tris-HCl, 0.15M NaCl pH 7.6

### **Imidazole solution**

Add 0.681g imidazole (Merck GPR 285466K) to 100ml distilled water. Store at 4°C.

0.1M imidazole solution

### **Diaminobenzidine (DAB) solution**

Add 180mg of 3,3'-diaminobenzidine tetrahydrochloride (Sigma D-5637) to 270ml distilled water, 30ml TBS, 1ml imidazole solution. Add 120µl hydrogen peroxide just before use.

### **1% Acid-Alcohol**

1400ml ethanol (Hayman 'Absolute Alcohol 100'), 580ml distilled water, 20ml concentrated HCl (Merck 'Analar' 10125QD)

## **Appendix 3 Epidemiology questionnaire**

Name

Address

Date of birth

### **SYMPTOMS**

1. When you first developed your symptoms, which of the following did you have and how bad were they on a scale of 1 – 10 with 10 being the worst? (please circle)

Burning	0 1 2 3 4 5 6 7 8 9 10
Itching	0 1 2 3 4 5 6 7 8 9 10
Pain at intercourse	0 1 2 3 4 5 6 7 8 9 10
Discharge	0 1 2 3 4 5 6 7 8 9 10
Tampon difficulty	0 1 2 3 4 5 6 7 8 9 10

2. With regard to your current symptoms, which of the following do you have and how bad are they on a scale of 1 – 10 with 10 being the worst? (please circle)

Burning	0 1 2 3 4 5 6 7 8 9 10
Itching	0 1 2 3 4 5 6 7 8 9 10
Pain at intercourse	0 1 2 3 4 5 6 7 8 9 10
Discharge	0 1 2 3 4 5 6 7 8 9 10
Tampon difficulty	0 1 2 3 4 5 6 7 8 9 10

3. Do you think anything precipitated your symptoms? If yes, please specify

4. Are your symptoms constant or intermittent?

5. When are your symptoms worse? Please circle

During period

Before period

After period

Mid cycle

No relation to period

6. What treatments have you had previously?

7. Have any of these treatments been successful? If so please specify.

## **CONTRACEPTION**

8. What are you currently using as contraception?
9. How long have you used this?
10. What have you used as contraception in the past?
11. Did your symptoms alter when you changed contraception? If yes please specify.

## **SEXUAL HISTORY**

12. How old were you when you first had sexual intercourse?
13. How many sexual partners have you had?
14. How long have you been with your current partner?
15. Did you have symptoms with a previous partner?
16. How many times per week do you have intercourse
17. If you are not having intercourse is this because it is too painful?

## **PERIODS**

18. When was your last period?
19. How long is your cycle and how long do they last?
20. Are your periods regular?
21. If you do not have periods any longer, did they used to be regular?
22. If your periods are regular, is this because you on taking the pill?
23. Are your periods painful?
24. Do you use tampons? If not, is this because of choice, or because it is too painful?

25. When was your last smear?

25. Have you ever had an abnormal smear? If so please specify.

### **YOUR PREGNANCIES**

26. Please state how many of the following you have had:

Children

Miscarriage

Termination

Ectopic

27. How many of the following deliveries have you had?

Normal

Caesarean

Forceps

Ventouse (suction)

28. Did you have a cut or a tear?

29. Did you have problems with your wound after delivery?

30. Did you breast feed? If yes, for how long?

31. How long after your baby did you resume sexual intercourse?

### **PREVIOUS MEDICAL PROBLEMS**

32. Have you had any illnesses or operations? Please specify.

33. Have you ever had any gynaecological problems? If yes please specify.

34. Have you ever had any of these infections?

Thrush

Chlamydia

Warts

Herpes

Trichomonas

Gonorrhoea

Bacterial Vaginosis

35. Do you have other skin problems?



## **GENERAL**

36. Does diet affect your symptoms?

37. Does anything run in the family?

38. Are you allergic to anything? If so please specify.

39. Is there anything else that you think is relevant to your symptoms?